PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

# PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

| | |
|---|---|
| **Date of mailing** (day/month/year)<br>06 September 2000 (06.09.00) | |
| **International application No.**<br>PCT/GB99/04129 | **Applicant's or agent's file reference**<br>40322/JMD |
| **International filing date** (day/month/year)<br>09 December 1999 (09.12.99) | **Priority date** (day/month/year)<br>10 December 1998 (10.12.98) |
| **Applicant**<br>DALGLEISH, Angus, George et al | |

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

07 July 2000 (07.07.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

| | |
|---|---|
| **The International Bureau of WIPO**<br>**34, chemin des Colombettes**<br>**1211 Geneva 20, Switzerland** | Authorized officer<br>Juan Cruz |
| Facsimile No.: (41-22) 740.14.35 | Telephone No.: (41-22) 338.83.38 |

PATENT COOPERATION TREATY

09/857691

# PCT

## INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| Applicant's or agent's file reference | FOR FURTHER ACTION | see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below. |
|---|---|---|
| 40322/JMD | | |
| International application No. | International filing date *(day/month/year)* | (Earliest) Priority Date *(day/month/year)* |
| PCT/GB 99/04129 | 09/12/1999 | 10/12/1998 |
| Applicant | | |
| ONYVAX LIMITED et al. | | |

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   USE OF HUMAN PROSTATE CELL LINES IN CANCER TREATMENT

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 5

   [X] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 99/04129

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 22 is directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/04129

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K35/12 //C12N5:08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 97 28255 A (LINEHAN W MARSTON ;US HEALTH (US); VOCKE CATHY D (US); BRIGHT ROBE) 7 August 1997 (1997-08-07)<br>page 3, line 15 -page 4, line 30<br>page 4, line 31 -page 5, line 1<br>page 11, line 8-24<br>page 12, line 11-13<br>page 12, line 28-31<br>page 13, line 1-18<br>page 13, line 23 -page 14, line 9<br>---<br>-/-- | 1,3,5,6, 12,16, 17,19-23 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 16 June 2000 | 23. 06. 00 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Mennessier, T |

3

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | ABLIN R J: "A retrospective and prospective overview of prostate-specific antigen." JOURNAL OF CANCER RESEARCH AND CLINICAL ONCOLOGY, (1997). VOL. 123, NO. 11-12, PP. 583-94. JOURNAL CODE: HL5. ISSN: 0171-5216., XP000915510 Innapharma Inc., Upper Saddle River, NJ 07458-1935, USA. page 590, right-hand column -page 591, left-hand column | 18 |
| A | VIEWEG J ET AL: "Immunotherapy of prostate cancer in the Dunning rat model: use of cytokine gene modified tumor vaccines." CANCER RESEARCH, (1994 APR 1) 54 (7) 1760-5. , XP002140267 the whole document | 1-23 |
| A | TJOA, BENJAMIN (1) ET AL: "In vitro propagated dendritic cells from prostate cancer patients as a component of prostate cancer immunotherapy." PROSTATE, (1995) VOL. 27, NO. 2, PP. 63-69. , XP000915509 the whole document | 1-23 |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 99/04129

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9728255 A | 07-08-1997 | AU 7749398 A | 24-02-2000 |
| | | CA 2245099 A | 07-08-1997 |
| | | EP 0877798 A | 18-11-1998 |

09/857691

15

PATENT COOPERATION TREATY



# PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference | FOR FURTHER ACTION | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| 40322/JMD | | |
| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
| PCT/GB99/04129 | 09/12/1999 | 10/12/1998 |
| International Patent Classification (IPC) or national classification and IPC<br>A61K39/00 | | |
| Applicant<br>ONYVAX LIMITED et al. | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 07/07/2000 | 02.01.2001 |
| Name and mailing address of the international preliminary examining authority:<br>European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Authorized officer<br>Mennessier, T<br>Telephone No. +49 89 2399 8687 |

Form PCT/IPEA/409 (cover sheet) (January 1994)

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/04129

## I. Basis of the r port

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
**Description, pages:**

| | | | | | |
|---|---|---|---|---|---|
| 1-12 | as originally filed | | | | |

**Claims, No.:**

| | | | | | |
|---|---|---|---|---|---|
| 1-22 | as received on | 16/11/2000 | with letter of | 13/11/2000 | |

**Drawings, sheets:**

| | |
|---|---|
| 1/9-9/9 | as originally filed |

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   *(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 21.

because:

   ☒ the said international application, or the said claims Nos. 21 (with respect to industrial applicability) relate to the following subject matter which does not require an international preliminary examination (*specify*): **see separate sheet**

   ☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

   Novelty (N) Yes: Claims 1-22

| | | | |
|---|---|---|---|
| | No: | Claims | |
| Inventive step (IS) | Yes: | Claims | 1-22 |
| | No: | Claims | |
| Industrial applicability (IA) | Yes: | Claims | 1-20,22 |
| | No: | Claims | |

2. Citations and explanations
**see separate sheet**

**VII. Certain defects in the international application**

The following defects in the form or contents of the international application have been noted:
**see separate sheet**

1. Document cited

Reference is made to the following document:

# D1: WO 97/28255

2. Comments with respect to item III

Claim 21 relates to a method of the human body by surgery, i.e., to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

3. Comments with respect to item V

a) Novelty (Article 33(2) PCT)

(i) None of the prior art documents cited in the international search report discloses the use in an allogeneic method of prophylaxis or treatment of prostate cancer of three prostate cell lines from different sources, at least one of them being from a normal tissue, i.e., from a normal prostate sample from a donor without prostate cancer.

(ii) Therefore, the claimed subject-matter as a whole can be regarded as new.

b) Inventive step (Article 33(3) PCT)

(i) Document D1 which is regarded as the closest state of the art discloses the use of cell lines which are deliberatly matched from the same prostate of the same patient in the treatment of prostate cancer.

(ii) It is considered that the person skilled in the art facing the technical problem posed by the provision of efficient therapeutic means efficient

at treating prostate cancer would not have been suggested by document D1, taken into consideration alone or in combination with any other of the cited prior art documents, that a combination of three prostate cell lines from different donors at least one being a normal cell might represent such a means useful in an allogeneic method of treatment.

(iii) Therefore, it can be further considered that the claimed subject-matter represents a non-obvious solution to the technical problem posed and, thereby, involves an inventive step.

c) Industrial applicability (Article 33(4 PCT)

(i) For the assessment of the present claim 21 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

(ii) The subject-matter of claims 1-20 and 22 may be considered to be susceptible of industrial applicability. Nevertheless, the afore-mentioned remark may also apply to it in view of the precise inventions to which said claims are directed ( **an agent for the treatment of prostate cancer** (claims 1-10), such an agent in combination with an adjuvant, (claims 1-19), **a composition for the treatment of prostate cancer**, (claim 20) and **the use of an agent [...] in the manufacture of a medicament** (claim 22).

4. Comments with respect to item VII

It has not been **convincingly** proved by the Applicant that each of the said cell

lines referred to in claims 5-8 had actually been made available to the public at the priority date claimed with regard to the present application. Therefore, the IPEA is doubtful about the sufficiency of the disclosure of the inventions as defined in the said claims, see futher Article 5 PCT.

## Claims

1. An allogeneic immunotherapeutic agent for the treatment of prostate cancer comprising three human prostate cell lines from three different sources, of which one, two or three cell lines are derived from normal tissue(s), wherein each said normal tissue(s) is (are) from a source which is a non-cancerous prostate.

2. An immunotherapeutic agent for the treatment of prostate cancer according to claim 1, comprising three human prostate cell lines of which one cell line is derived from normal tissue and the other two cell lines are derived from tumour tissues.

3. An immunotherapeutic agent for the treatment of prostate cancer according to claim 1, comprising three human prostate cell lines of which two cell lines are derived from normal tissue and the other cell line is derived from a tumour tissue.

4. An immunotherapeutic agent of claims 1, 2 and 3 where the lines derived from normal tissue are chosen from from PNT1A (ECACC Ref No: 95012614) or PNT2 (ECACC Ref No: 95012613)

5. An immunotherapeutic agent of claims 1, 2 and 3 where the line(s) derived from tumour tissue is/are chosen from NIH1519-CPTX, NIH1532-CP2TX, NIH1535-CP1TX, NIH1542-CP3TX, CA-HPV-10, LnCap, DU145 or PC3.

6. An immunotherapeutic agent for the treatment of prostate cancer comprising three cell lines, namely PNT2, NIH1542-CP3TX andDU145.

7. An immunotherapeutic agent for the treatment of prostate cancer comprising three cell lines, namely PNT2, NIH1542-CP3TX and LnCap.

8. An immunotherapeutic agent for the treatment of prostate cancer comprising three cell lines, namely PNT2, DU145 and LnCap.

9. An immunotherapeutic agent of claims 1-8 wherein the tumour cell lines have been irradiated at 50 to 300 Gy.

10. An immunotherapeutic agent of claims 1-8 wherein the tumour cell lines have been irradiated at 100 to 150 Gy.

11. An allogeneic immunogenic composition comprising an immunotherapeutic agent of claims 1-10 combined with a vaccine adjuvant selected from mycobacterial preparations such as BCG or M. Vaccae, Tetanus toxoid, Diphtheria toxoid, Bordetella Pertussis, interleukin 2, interleukin 12, interleukin 4, interleukin 7, Complete Freund's Adjuvant, Incomplete Freund's Adjuvant or other non-specific agents adjuvant.

12. An immunogenic composition comprising an immunotherapeutic agent of claims 1-10 combined with a vaccine adjuvant selected from mycobacterial preparations such as BCG or M. Vaccae.

13. An immunotherapeutic agent or composition of claims 1-12 wherein the cells are formulated with a cryoprotectant solution including but not limited to 10-30% v/v

aqu ous glycerol solution, 5-20% v/v dimethyl sulphoxide or 5-20% w/v human serum albumin either as single cryoprotectants or in combination.

14. An immunotherapeutic agent or composition of claims 1-12 wherein the cells are formulated with a cryoprotectant solution including 5-20% v/v dimethyl sulphoxide and 5-20% w/v human serum albumin in combination.

15. An immunotherapeutic agent or composition of claims 1-14 that induces an immune response in patients characterised by activation of immune T-cells.

16. An immunotherapeutic agent or composition of claims 1-14 that induces an immune response in patients characterised by induction of antibody production.

17. An immunotherapeutic agent or composition of claims 1-14 that induces a decrease in the rate of rise or a decline in the level of serum PSA in prostate cancer patients.

18. An immunotherapeutic agent or composition according to claims 1 to 17 that is administered intradermally.

19. An immunotherapeutic agent or composition according to claims 1 to 17 that is administered intra-prostatically.

20. An allogeneic immunotherapeutic vaccine composition for the treatment of prostate cancer, which comprises or consists of an agent according to any preceding claim together with a physiologically acceptable excipient, adjuvant or carrier.

21. An allogeneic method of prophylaxis or treatment of prostate cancer, which includes administering to a patient an agent or composition according to any preceding claim in one or more doses in suitable dosage form.

22. Use of an agent according to any of claims 1 to 10 in the manufacture of a medicament for the allogeneic treatment of human prostate cancer.

Claims

1. An immunotherapeutic agent for the treatment of prostate cancer comprising three human prostate cell lines of which one cell line is derived from normal tissue and the other two cell lines are derived from tumour tissues.

2. An immunotherapeutic agent for the treatment of prostate cancer comprising three human prostate tumour cell lines of which one cell line is derived from a primary tumour and the other two cell lines are derived from two different tumour tissues.

3. An immunotherapeutic agent for the treatment of prostate cancer comprising three human prostate cell lines of which three cell lines are derived from one, two or three normal tissue(s).

4. An immunotherapeutic agent for the treatment of prostate cancer comprising three human prostate cell lines of which two cell lines are derived from normal tissue and the other cell line is derived from a tumour site.

5. An immunotherapeutic agent of claims 1, 3 and 4 where the lines derived from normal tissue are chosen from from PNT1A (ECACC Ref No: 95012614) or PNT2 (ECACC Ref No: 95012613)

6. An immunotherapeutic agent of claims 1, 2 and 4 where the line(s) derived from tumour tissue is/are chosen from NIH1519-CPTX, NIH1532-CP2TX, NIH1535-CP1TX, NIH1542-CP3TX, CA-HPV-10, LnCap, DU145 or PC3.

7. An immunotherapeutic agent for the treatment of prostate cancer comprising three cell lines, namely PNT2, NIH1542-CP3TX andDU145.

8. An immunotherapeutic agent for the treatment of prostate cancer comprising three cell lines, namely PNT2, NIH1542-CP3TX and LnCap.

9. An immunotherapeutic agent for the treatment of prostate cancer comprising three cell lines, namely PNT2, DU145 and LnCap.

10. An immunotherapeutic agent of claims 1-9 wherein the tumour cell lines have been irradiated at 50 to 300 Gy.

11. An immunotherapeutic agent of claims 1-9 wherein the tumour cell lines have been irradiated at 100 to 150 Gy.

12. An immunogenic composition comprising an immunotherapeutic agent of claims 1-11 combined with a vaccine adjuvant selected from mycobacterial preparations such as BCG or M. Vaccae, Tetanus toxoid, Diphtheria toxoid, Bordetella Pertussis, interleukin 2, interleukin 12, interleukin 4, interleukin 7, Complete Freund's Adjuvant, Incomplete Freund's Adjuvant or other non-specific agents adjuvant.

13. An immunogenic composition comprising an immunotherapeutic agent of claims 1-11 combined with a vaccine adjuvant selected from mycobacterial preparations such as BCG or M. Vaccae.

14. An immunotherapeutic agent or composition of claims 1-13 wherein the cells are formulated with a cryoprotectant solution including but not limited to 10-30% v/v aqueous glycerol solution, 5-20% v/v dimethyl sulphoxide or 5-20% w/v human serum albumin either as single cryoprotectants or in combination.

15. An immunotherapeutic agent or composition of claims 1-13 wherein the cells are formulated with a cryoprotectant solution including 5-20% v/v dimethyl sulphoxide and 5-20% w/v human serum albumin in combination.

16. An immunotherapeutic agent or composition of claims 1-15 that induces an immune response in patients characterised by activation of immune T-cells.

17. An immunotherapeutic agent or composition of claims 1-15 that induces an immune response in patients characterised by induction of antibody production.

18. An immunotherapeutic agent or composition of claims 1-15 that induces a decrease in the rate of rise or a decline in the level of serum PSA in prostate cancer patients.

19. An immunotherapeutic agent or composition according to claims 1 to 18 that is administered intradermally.

20. An immunotherapeutic agent or composition according to claims 1 to 18 that is administered intra-prostatically.

21. A immunotherapeutic vaccine composition for the treatment of prostate cancer, which comprises or consists of an agent according to any preceding claim together with a physiologically acceptable excipient, adjuvant or carrier.

22. A method of prophylaxis or treatment of prostate cancer, which includes administering to a patient an agent or composition according to any preceding claim in one or more doses in suitable dosage form.

23. Use of an agent according to any of claims 1 to 11 in the manufacture of a medicament for the treatment of human prostate cancer.

**PCT** WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
A61K 35/12 // C12N 5:08

A3

(11) International Publication Number: **WO 00/33869**

(43) International Publication Date: 15 June 2000 (15.06.00)

(21) International Application Number: PCT/GB99/04129

(22) International Filing Date: 9 December 1999 (09.12.99)

(30) Priority Data:
9827104.2 10 December 1998 (10.12.98) GB

(71) Applicant *(for all designated States except US)*: ONYVAX LIMITED [GB/GB]; St. Georges Hospital Medical School, Cranmer Terrace, P.O. Box 17717, London SW17 0WG (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: DALGLEISH, Angus, George [GB/GB]; Onyvax Limited, St. Georges Hospital Medical School, Cranmer Terrace, P.O. Box 17717, London SW17 0WG (GB). SMITH, Peter, Michael [GB/GB]; Onyvax Limited, St. Georges Hospital Medical School, Cranmer Terrace, P.O. Box 17717, London SW17 0WG (GB). SUTTON, Andrew, Derek [GB/GB]; Onyvax Limited, St. Georges Hospital Medical School, Cranmer Terrace, P.O. Box 17717, London SW17 0WG (GB). WALKER, Anthony, Ian [GB/GB]; Onyvax Limited, St. Georges Hospital Medical School, Cranmer Terrace, P.O. Box 17717, London SW17 0WG (GB).

(74) Agent: DAVIES, Jonathan, Mark; Reddie & Grose, 16 Theobalds Road, London WCLX 8PL (GB).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

**Published**
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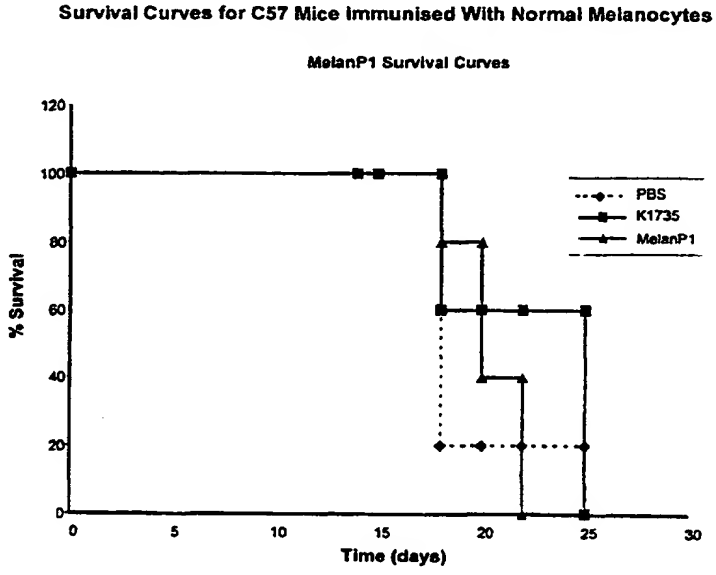
12 October 2000 (12.10.00)

(54) **Title:** USE OF HUMAN PROSTRATE CELL LINES IN CANCER TREATMENT

(57) **Abstract**

The invention here relates to a product comprised of a cell line or lines intended for use as an allogeneic immunotherapy agent for the treatment of cancer in mammals and humans. All of the studies of cell–based cancer vaccines to date have one feature in common, namely the intention to use cells that contain at least some TSAs and/or TAAs that are shared with the antigens present in patients' tumour. In each case, tumour cells are utilised as the starting point on the premise that only tumour cells will contain TSAs or TAAs of relevance, and the tissue origins of the cells are matched to the tumour site in patients. A primary aspect of the invention is the use of immortalised normal, non–malignant cells as the basis of an allogeneic cell cancer vaccine. Normal cells do not possess TSAs or relevant concentrations of TAAs and hence it is surprising that normal cells are effective as anti–cancer vaccines. For prostate cancer, for example, a vaccine may be based on one or a combination of different immortalised normal cell lines derived from the prostate. The cell lines are lethally irradiated utilising gamma irradiation at 50–300 Gy to ensure that they are replication incompetent prior to use in the mammal or human.



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# INTERNATIONAL SEARCH REPORT

Interr ›nal Application No
PCT/GB 99/04129

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K35/12 //C12N5:08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 97 28255 A (LINEHAN W MARSTON ;US HEALTH (US); VOCKE CATHY D (US); BRIGHT ROBE) 7 August 1997 (1997-08-07)<br>page 3, line 15 -page 4, line 30<br>page 4, line 31 -page 5, line 1<br>page 11, line 8-24<br>page 12, line 11-13<br>page 12, line 28-31<br>page 13, line 1-18<br>page 13, line 23 -page 14, line 9<br>---<br>-/-- | 1,3,5,6, 12,16, 17,19-23 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 16 June 2000 | 23. 06. 00 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Mennessier, T |

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# INTERNATIONAL SEARCH REPORT

Inter ›nal Application No
PCT/GB 99/04129

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | ABLIN R J: "A retrospective and prospective overview of prostate-specific antigen." JOURNAL OF CANCER RESEARCH AND CLINICAL ONCOLOGY, (1997). VOL. 123, NO. 11-12, PP. 583-94. JOURNAL CODE: HL5. ISSN: 0171-5216., XP000915510 Innapharma Inc., Upper Saddle River, NJ 07458-1935, USA. page 590, right-hand column -page 591, left-hand column | 18 |
| A | VIEWEG J ET AL: "Immunotherapy of prostate cancer in the Dunning rat model: use of cytokine gene modified tumor vaccines." CANCER RESEARCH, (1994 APR 1) 54 (7) 1760-5. , XP002140267 the whole document | 1-23 |
| A | TJOA, BENJAMIN (1) ET AL: "In vitro propagated dendritic cells from prostate cancer patients as a component of prostate cancer immunotherapy." PROSTATE, (1995) VOL. 27, NO. 2, PP. 63-69. , XP000915509 the whole document | 1-23 |

3

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 99/04129

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 22 is directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

..ormation on patent family members

Intern nal Application No
PCT/GB 99/04129

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 9728255 A | 07-08-1997 | AU 7749398 A | 24-02-2000 |
| | | CA 2245099 A | 07-08-1997 |
| | | EP 0877798 A | 18-11-1998 |

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(72) Inventors; and

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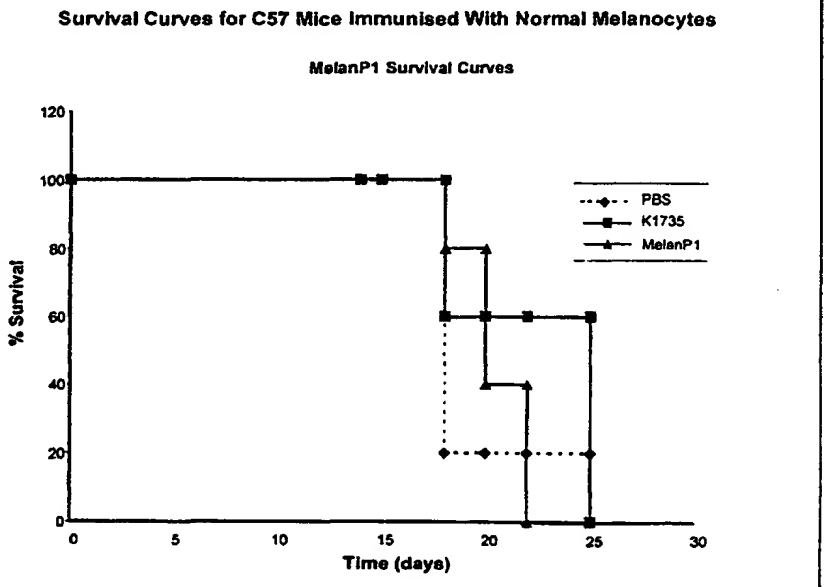
**Published**

*Without international search report and to be republished upon receipt of that report.*

(54) Title: NEW CANCER TREATMENTS

(57) Abstract

The invention here relates to a product comprised of a cell line or lines intended for use as an allogeneic immunotherapy agent for the treatment of cancer in mammals and humans. All of the studies of cell–based cancer vaccines to date have one feature in common, namely the intention to use cells that contain at least some TSAs and/or TAAs that are shared with the antigens present in patients' tumour. In each case, tumour cells are utilised as the starting point on the premise that only tumour cells will contain TSAs or TAAs of relevance, and the tissue origins of the cells are matched to the tumour site in patients. A primary aspect of the invention is the use of immortalised normal, non–malignant cells as the basis of an allogeneic cell cancer vaccine. Normal cells do not possess TSAs or relevant concentrations of TAAs and hence it is surprising that normal cells are effective as anti–cancer vaccines. For prostate cancer, for example, a vaccine may be based on one or a combination of different immortalised normal cell lines derived from the prostate. The cell lines are lethally irradiated utilising gamma irradiation at 50–300 Gy to ensure that they are replication incompetent prior to use in the mammal or human.



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# New Cancer Treatments

## Field of the Invention

This invention is concerned with agents for the treatment of primary, metastatic and residual cancer in mammals (including humans) by inducing the immune system of the mammal or human afflicted with cancer to mount an attack against the tumour lesion. In particular, the invention pertains to the use of whole-cells, derivatives and portions thereof with or without vaccine adjuvants and/or other accessory factors. More particularly, this disclosure describes the use of particular combinations of whole-cells and derivatives and portions thereof that form the basis of treatment strategy.

## Background to the Invention

It is known in the field that cancerous cells contain numerous mutations, qualitative and quantitative, spatial and temporal, relative to their normal, non-cancerous counterparts and that at certain periods during tumour cells' growth and spread a proportion of these are capable of being recognised by the hosts' immune system as abnormal. This has led to numerous research efforts world-wide to develop immunotherapies that harness the power of the hosts' immune system and direct it to attack the cancerous cells, thereby eliminating such aberrant cells at least to a level that is not life-threatening (reviewed in Maraveyas, A. & Dalgleish, A.G. 1977 *Active immunotherapy for solid tumours in vaccine design* in The Role of Cytokine Networks, Ed. Gregoriadis *et al.*, Plenum Press, New York, pages 129-145; Morton, D.L. and Ravindranath, M.H. 1996 *Current concepts concerning melanoma vaccines* in Tumor Immunology – Immunotherapy and Cancer Vaccines, ed. Dalgleish, A.G. and Browning, M., Cambridge University Press, pages 241-268. See also other papers in these publications for further detail).

Numerous approaches have been taken in the quest for cancer immunotherapies, and these can be classified under five categories:

*Non-specific immunotherapy*

Efforts to stimulate the immune system non-specifically date back over a century to the pioneering work of William Coley (Coley, W.B., 1894 Treatment of inoperable malignant tumours with toxins of erisipelas and the Bacillus prodigosus. Trans. Am. Surg. Assoc. 12: 183). Although successful in a limited number of cases (e.g. BCG for the treatment of urinary bladder cancer, IL-2 for the treatment of melanoma and renal cancer) it is widely acknowledged that non-specific immunomodulation is unlikely to prove sufficient to treat the majority of cancers. Whilst non-specific immune-stimulants may lead to a general enhanced state of immune responsiveness, they lack the targeting capability and also subtlety to deal with tumour lesions which have many mechanisms and plasticity to evade, resist and subvert immune-surveillance.

*Antibodies and monoclonal antibodies*

Passive immunotherapy in the form of antibodies, and particularly monoclonal antibodies, has been the subject of considerable research and development as anti-cancer agents. Originally hailed as the magic bullet because of their exquisite specificity, monoclonal antibodies have failed to live up to their expectation in the field of cancer immunotherapy for a number of reasons including immune responses to the antibodies themselves (thereby abrogating their activity) and

inability of the antibody to access the lesion through the blood vessels. To date, three products have been registered as pharmaceuticals for human use, namely *Panorex* (Glaxo-Wellcome), *Rituxan* (IDEC/Genentech/Hoffman la Roche) and *Herceptin* (Genentech/Hoffman la Roche) with over 50 other projects in the research and development pipeline. Antibodies may also be employed in active immunotherapy utilising anti-idiotype antibodies which appear to mimic (in an immunological sense) cancer antigens. Although elegant in concept, the utility of antibody-based approaches may ultimately prove limited by the phenomenon of 'immunological escape' where a subset of cancer cells in a mammalian or human subject mutates and loses the antigen recognised by the particular antibody and thereby can lead to the outgrowth of a population of cancer cells that are no longer treatable with that antibody.

*Subunit vaccines*

Drawing on the experience in vaccines for infectious diseases and other fields, many researchers have sought to identify antigens that are exclusively or preferentially associated with cancer cells, namely tumour specific antigens (TSA) or tumour associated antigens (TAA), and to use such antigens or fractions thereof as the basis for specific active immunotherapy.

There are numerous ways to identify proteins or peptides derived therefrom which fall into the category of TAA or TSA. For example, it is possible to utilise differential display techniques whereby RNA expression is compared between tumour tissue and adjacent normal tissue to identify RNAs which are exclusively or preferentially expressed in the lesion. Sequencing of the RNA has identified several TAA and TSA which are expressed in that specific tissue at that specific time, but therein lies the potential deficiency of the approach in that identification of the TAA or TSA represents only a "snapshot" of the lesion at any given time which may not provide an adequate reflection of the antigenic profile in the lesion over time. Similarly a combination of cytotoxic T lymphocyte (CTL) cloning and expression-cloning of cDNA from tumour tissue has lead to identification of many TAA and TSA, particularly in melanoma. The approach suffers from the same inherent weakness as differential display techniques in that identification of only one TAA or TSA may not provide an appropriate representation of a clinically relevant antigenic profile.

Over fifty such subunit vaccine approaches are in development for the treatment of a wide range of cancers, although none has yet received marketing authorisation for use as a human pharmaceutical product. In a similar manner to that described for antibody-based approaches above, subunit vaccines may also be limited by the phenomenon of immunological escape.

*Gene therapy*

The majority of gene therapy trials in human subjects have been in the area of cancer treatment, and of these a substantial proportion have been designed to trigger and/or amplify patients' immune responses. Of particular note in commercial development are Allovectin-7 and Leuvectin, being developed by Vical Inc for a range of human tumours, CN706 being developed by Calydon Inc for the treatment of prostate cancer, and StressGen Inc.'s stress protein gene therapy for melanoma and lung cancer. At the present time, it is too early to judg wheth r these and the many other 'immuno-gen therapies' in development by commercial and academic bodies will ultimat ly prove successful, but it is widely accepted that commercial utility of these approaches are likely to be more than a decade away.

*Cell-based vaccines*

Tumours have the remarkable ability to counteract the immune system in a variety of ways including: downregulation of the expression of potential target proteins; mutation of potential target proteins; downregulation of surface expression of receptors and other proteins; downregulation of MHC class I and II expression thereby disallowing direct presentation of TAA or TSA peptides; downregulation of co-stimulatory molecules leading to incomplete stimulation of T-cells leading to anergy; shedding of selective, non representative membrane portions to act as decoy to the immune system; shedding of selective membrane portions to anergise the immune system; secretion of inhibitory molecules; induction of T-cell death; and many other ways. What is clear is that the immunological heterogeneity and plasticity of tumours in the body will have to be matched to a degree by immunotherapeutic strategies which similarly embody heterogeneity. The use of whole cancer cells, or crude derivatives thereof, as cancer immunotherapies can be viewed as analogous to the use of whole inactivated or attenuated viruses as vaccines against viral disease. The potential advantages are:

(a) whole cells contain a broad range of antigens, providing an antigenic profile of sufficient heterogeneity to match that of the lesions as described above;

(b) being multivalent (i.e. containing multiple antigens), the risk of immunological escape is reduced (the probability of cancer cells 'losing' all of these antigens is remote); and

(c) cell-based vaccines include TSAs and TAAs that have yet to be identified as such; it is possible if not likely that currently unidentified antigens may be clinically more relevant than the relatively small number of TSAs/TAAs that are known.

Cell-based vaccines fall into two categories. The first, based on autologous cells, involves the removal of a biopsy from a patient, cultivating tumour cells *in vitro*, modifying the cells through transfection and/or other means, irradiating the cells to render them replication-incompetent and then injecting the cells back into the same patient as a vaccine. Although this approach enjoyed considerable attention over the past decade, it has been increasingly apparent that this individually-tailored therapy is inherently impractical for several reasons. The approach is time consuming (often the lead time for producing clinical doses of vaccine exceeds the patients' life expectancy), expensive and, as a 'bespoke' product, it is not possible to specify a standardised product (only the procedure, not the product, can be standardised and hence optimised and quality controlled). Furthermore, the tumour biopsy used to prepare the autologous vaccine will have certain growth characteristics, interactions and communication with surrounding tissue that makes it somewhat unique. This alludes to a potentially significant disadvantage to the use of autologous cells for immunotherapy: a biopsy which provides the initial cells represents an immunological snapshot of the tumour, in that environment, at that point in time, and this may be inadequate as an immunological representation over time for the purpose of a vaccine with sustained activity that can be given over the entire course of the disease.

The second type of cell-based vaccine and the subject of the current invention describes the use of allogeneic cells which are be genetically (and hence immunologically) mismatched to the patients. Allogeneic cells benefit from the same advantag s of multivalency as autologous cells. In addition, as allogeneic cell

vaccines can be based on immortalised cell lines which can be cultivated indefinitely *in vitro,* thus this approach does not suffer the lead-time and cost disadvantages of autologous approaches. Similarly the allogeneic approach offers the opportunity to use combinations of cells types which may match the disease profile of an individual in terms of stage of the disease, the location of the lesion and potential resistance to other therapies.

There are numerous published reports of the utility of cell-based cancer vaccines (see, for example, Dranoff, G. *et al.* WO 93/06867; Gansbacher, P. WO 94/18995; Jaffee, E.M. *et al.* WO 97/24132; Mitchell, M.S. WO 90/03183; Morton, D.M. *et al.* WO 91/06866). These studies encompass a range of variations from the base procedure of using cancer cells as an immunotherapy antigen, to transfecting the cells to produce GM-CSF, IL-2, interferons or other immunologically-active molecules and the use of 'suicide' genes. Groups have used allogeneic cell lines that are HLA-matched or partially-matched to the patients' haplotype and also allogeneic cell lines that are mismatched to the patients' haplotype in the field of melanoma and also mismatched allogeneic prostate cell lines transfected with GM-CSF.

**Description of the Invention**

The invention disclosed here relates to a product comprised of a cell line or lines intended for use as an allogeneic immunotherapy agent for the treatment of cancer in mammals and humans.

All of the studies of cell-based cancer vaccines to date have one feature in common, namely the intention to use cells that contain at least some TSAs and/or TAAs that are shared with the antigens present in patients' tumour. In each case, tumour cells are utilised as the starting point on the premise that only tumour cells will contain TSAs or TAAs of relevance, and the tissue origins of the cells are matched to the tumour site in patients.

A primary aspect of the invention is the use of immortalised normal, non-malignant cells as the basis of an allogeneic cell cancer vaccine. Normal cells do not posses TSAs or relevant concentrations of TAAs and hence it is surprising that normal cells as described herein are effective as anti-cancer vaccines. The approach is general and can be adapted to any mammalian tumour by the use of immortalised normal cells derived from the same particular tissue as the tumour intended to be treated. Immortalised normal cells can be prepared by those skilled in the art using published methodologies, or they can be sourced from cell banks such as ATCC or ECACC, or they are available from several research groups in the field.

For prostate cancer, for example, a vaccine may be based on one or a combination of different immortalised normal cell lines derived from the prostate which can be prepared using methods reviewed and cited in Rhim, J.S. and Kung, H-F., 1997 Critical Reviews in Oncogenesis 8(4):305-328 or selected from PNT1A (ECACC Ref No: 95012614), PNT2 (ECACC Ref No: 95012613) or PZ-HPV-7 (ATCC Number: CRL-2221).

A further aspect of th invention is the addition of TSAs and/or TAAs by combining one or more immortalis d normal c ll line(s) with one, two or thre different cell lines

derived from primary or metastatic cancer biopsies.

All the appropriate cell lines will show good growth in large scale cell culture and sufficient characterisation to allow for quality control and reproducible production.

The cell lines are lethally irradiated utilising gamma irradiation at 50-300 Gy to ensure that they are replication incompetent prior to use in the mammal or human.

The cell lines and combinations referenced above, to be useful as immunotherapy agents must be frozen to allow transportation and storage, therefore a further aspect of the invention is any combination of cells referenced above formulated with a cryoprotectant solution. Suitable cryoprotectant solutions may include but are not limited to, 10-30% v/v aqueous glycerol solution, 5-20% v/v dimethyl sulphoxide or 5-20% w/v human serum albumin may be used either as single cryoprotectants or in combination.

A further embodiment of the invention is the use of the cell line combinations with non-specific immune stimulants such as BCG or M. Vaccae, Tetanus toxoid, Diphtheria toxoid, Bordetella Pertussis, interleukin 2, interleukin 12, interleukin 4, interleukin 7, Complete Freund's Adjuvant, Incomplete Freund's Adjuvant or other non-specific agents known in the art. The advantage is that the general immune stimulants create a generally enhanced immune status whilst the combinations of cell lines, both add to the immune enhancement through their haplotype mismatch and target the immune response to a plethora of TAA and TSA as a result of the heterogeneity of their specific origins.

The invention will now be described with reference to the following examples, and the Figures in which:

Figure 1 shows T-cell proliferation data for patients 112, 307, and 406;

Figure 2 shows Western Blot analysis of serum from patients 115, 304 and 402;

Figure 3 shows antibody titres of serum from patients 112, 305 and 402;

Figure 4 shows PSA data for patients 110, 303 and 404; and

Figure 5 shows survival curves for C57 mice immunised with normal melanocytes.

**Example 1**

***Growth, irradiation, formulation and storage of cells***

An immortalised cell line derived from normal prostate tissue namely PNT2 was grown in roller bottle culture in RPMI 1640 medium supplemented with 2 mM L-glutamine and 5% foetal calf serum (FCS) following recovery from liquid nitrogen stocks. Following expansion in T175 static flasks the cells were seeded into roller bottles with a growth surface area of 850 $cm^2$ at 1-20 $x10^7$ cells per roller bottle

An immortalised cell line derived from primary prostate tissue nam ly NIH1542-CP3TX was grown in roller bottle culture in KSFM media supplemented with 25 μg/ml bovine pituitary extract, 5 ng/ml of epidermal growth factor, 2 mM L-glutamine, 10 mM HEPES buffer and 5% foetal calf serum (FCS) (hereinafter called "modified

KSFM") following recovery from liquid nitrogen stocks. Following expansion in T175 static flasks the cells were seeded into roller bottles with a growth surface area of 1,700 $cm^2$ at 2-5 $x10^7$ cells per roller bottle

Two secondary derived cell lines were also used, namely LnCap and Du145 both of which were sourced from ATCC. LnCap was grown in large surface area static flasks in RPMI media supplemented with 10% FCS and 2 mM L-glutamine following seeding at 1-$10x10^6$ cells per vessel and then grown to near confluence. Du-145 was expanded from frozen stocks in static flasks and then seeded into 850 $cm^2$ roller bottles at 1-$20x10^7$ cells per bottle and grown to confluence in DMEM medium containing 10% FCS and 2 mM L-glutamine. All cell lines were harvested utilising trypsin at 1x normal concentration. Following extensive washing in DMEM the cells were re-suspended at a concentration of 5-$40x10^6$ cells/ml and irradiated at 50-300 Gy using a $Co^{60}$ source. Following irradiation the cells were formulated in cryopreservation solution composing of 10% DMSO, 8% human serum albumin in phosphate buffered saline, and frozen at a cell concentration of 5-150 $x10^6$ cells/ml, in liquid nitrogen until required for use.

***Vaccination***

Prostate cancer patients were selected on the basis of being refractory to hormone therapy with a serum PSA level of at least 30 ng/ml. Ethical permission and MCA (UK Medicines Control Agency) authorization were sought and obtained to conduct this trial.

One of three vaccination schedules was followed for each arm of the trial:

| | **Cell Lines Administered** | | |
|---|---|---|---|
| **Dose** | **Trial Arm A** | **Trial Arm B** | **Trial Arm C** |
| 1,2 and 3 | PNT2 | Du145 | LnCap |
| 4 and subsequent | PNT2 / Du145/ NIH1542 | PNT2 / Du145/ LnCap | PNT2 / NIH1542/ LnCap |

The cells were warmed gently in a water bath at 37 °C and admixed with mycobacterial adjuvant prior to injection into patients. Injections were made intra-dermally at four injection sites into draining lymph node basins. The minimum interval between doses was two weeks, and most of the doses were given at intervals of four weeks. Prior to the first dose, and prior to some subsequent doses, the patients were tested for delayed-type hypersensitivity (DTH) against the four cell lines listed in the vaccination schedule above (all tests involved 0.8 x $10^6$ cells with no adjuvant).

***Analysis of Immunological Response***

*(a) T-Cell Proliferation Responses*

To determine if vaccination resulted in a specific expansion of T-cell populations that recognised antigens derived from th vaccinating c ll lines we perform d a proliferation assay on T-cells following stimulation with lysates of the prostate cell

lin s. Whole blood was extracted at each visit to the clinic and used in a BrdU (bromodeoxyuridine) based proliferation assay as described below:

*Patient BrdU proliferation method*

*Reagents*

| | | |
|---|---|---|
| RPMI | | Life Technologies, Paisley Scotland. |
| BrdU | | Sigma Chemical Co, Poole, Dorset. |
| PharMlyse | 35221E | Pharmingen, Oxford UK |
| Cytofix/Cytoperm | 2090KZ | " |
| Perm/Wash buffer (x10) | 2091KZ | " |
| FITC Anti-BrdU/Dnase | 340649 | Becton Dickinson |
| PerCP Anti-CD3 | 347344 | " |
| Pe Anti-CD4 | 30155X | Pharmingen |
| Pe Anti-CD8 | 30325X | " |
| FITC mu-IgG1 | 349041 | Becton Dickinson |
| PerCP IgG1 | 349044 | " |
| PE IgG1 | 340013 | " |

**Method**

1) Dilute 1 ml blood with 9 ml RPMI + 2mM L-gln +PS +50$\mu$M 2-Me. Do not add serum. Leave overnight at 37°C
2) On following morning, aliquot 450$\mu$l of diluted blood into wells of a 48-well plate and add 50$\mu$l of stimulator lysate. The lysate is made by freeze-thawing tumour cells ($2x10^6$ cell equivalents/ml) x3 in liquid nitrogen and then storing aliquots frozen until required.
3) Culture cells at 37°C for 5 days
4) On the evening of day 5 add 50$\mu$l BrdU @ 30$\mu$g/ml
5) Aliquot 100$\mu$l of each sample into a 96-well round-bottomed plate.
6) Spin plate and discard supernatant
7) Lyse red cells using 100$\mu$l *Pharmlyse* for 5minutes at room temperature
8) Wash x2 with 50$\mu$l of Cytofix
9) Spin and remove supernatant by flicking
10) Permeabilise with 100$\mu$l Perm wash for 10mins at RT
11) Add 30$\mu$l of antibody mix comprising antibodies at correct dilution made up to volume with Perm-wash
12) Incubate for 30 mins in the dark at room temperature.
13) Wash x1 and resuspend in 100$\mu$l 2% paraformaldehyde
14) Add this to 400$\mu$l FACSFlow in cluster tubes ready for analysis
15) Analyse on FACScan, storing 3000 gated CD3 events.

**6-well plate for stimulation**

| | Nil | ConA | 1542 | LnCap | Du145 | Pnt2 |
|---|---|---|---|---|---|---|
| PBL 1 | | | | | | |
| PBL 2 | | | | | | |
| PBL 3 | | | | | | |
| PBL 4 | | | | | | |
| PBL 5 | | | | | | |
| PBL 6 | | | | | | |

**96-well plate for antibody staining**

| PBL 1 | | PBL 2 | | PBL 3 | | PBL 4 | | PBL 5 | | PBL 6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Nil A | 15 D | Nil A | 15 D | Nil A | 15 D | Nil A | 15 D | Nil A | 15 D | Nil A | 15 D |
| Nil D | 15 E | Nil D | 15 E | Nil D | 15 E | Nil D | 15 E | Nil D | 15 E | Nil D | 15 E |
| Nil E | Ln D | Nil E | Ln D | Nil E | Ln D | Nil E | Ln D | Nil E | Ln D | Nil E | Ln D |
| Con D | Ln E | Con D | Ln E | Con D | Ln E | Con D | Ln E | Con D | Ln E | Con D | Ln E |
| Con E | Du D | Con E | Du D | Con E | Du D | Con E | Du D | Con E | Du D | Con E | Du D |
| | Du E | | Du E | | Du E | | Du E | | Du E | | Du E |
| | Pn D | | Pn D | | Pn D | | Pn D | | Pn D | | Pn D |
| | Pn E | | Pn E | | Pn E | | Pn E | | Pn E | | Pn E |

Legend:

A: IgG1-FITC (5μl) IgG1-PE (5μl) IgG1-PerCP (5μl)
15μlMoAb+15μl

D: BrdU-FITC (5μl) CD4-PE (5μl) CD3-PerCP (5μl)
15μlMoAb+15μl

E: BrdU-FITC (5μl) CD8-PE (5μl) CD3-PerCP (5μl)
15μlMoAb+15μl

15: NIH1542-CP3TX

Ln: LnCap

D: Du145

Pn: PNT2

Con: ConA lectin (positive control)

Nil: No stimulation

The results for the proliferation assays are shown in Figure 1 where a proliferation index for either CD4 or CD8 positive T-cells are plotted against the various cell lysates. The proliferation index being derived by dividing through the percentage of T-cells proliferating by the no-lysate control.

Results are shown for patient numbers 112, 307 and 406. Results are given for four cell lysates namely, NIH1542, LnCap, DU-145 and PNT-2. Overall, 50% of patients treated mount a specific proliferative response to at least one of the cell lines.

*(b) Western Blots Utilising Patients' Serum*

Standardised cell lysates were prepared for a number of prostate cell lines to enable similar quantities of protein to be loaded on a denaturing SDS PAGE gel for Western blot analysis. Each blot was loaded with molecular weight markers, and equal amounts of protein derived from cell lysates of NIH1542, LnCap, DU-145 and PNT-2. The blot was then probed with serum from patients derived from pre-vaccination and following 16 weeks vaccination (four to six doses).

*Method*

a) Sample Preparation (Prostate Tumor Lines)

- Wash cell pellets 3 times in PBS
- Re-suspend at 1 x $10^7$ cells/ml of lysis buffer
- Pass through 5 cycles of rapid freeze thaw lysis in liquid nitrogen/water bath
- Centrifuge at 1500 rpm for 5 min to remove cell debris
- Ultracentrifuge at 20,000 rpm for 30 min to remove membrane contaminants
- Aliquot at 200 μl and stored at -80°C

b) Gel Electrophoresis

- Lysates mixed 1:1 with Laemelli sample buffer and boiled for 5 min
- 20 μg samples loaded into 4-20% gradient gel wells
- Gels run in Bjerrum and Schafer-Nielson transfer buffer (with SDS) at 200 V for 35 min.

c) Western Transfer

- Gels, nitrocellulose membranes and blotting paper equilibrated in transfer buffer for 15 min
- Arrange gel-nitrocellulose sandwich on anode of semi-dry electrophoretic transfer cell: 2 sheets of blotting paper, nitrocellulose membrane, gel, 2 sheets of blotting paper
- Apply cathode and run at 25 V for 90 min.

d) Immunological Detection of Proteins

- Block nitrocellulose membranes overnight at 4°C with 5% Marvel in PBS/0/05% Tween 20
- Rinse membranes twice in PBS/0.05% Tween 20, then wash for 20 min and 2 x 5 min at RT on a shaking platform
- Incubate membranes in 1:20 dilution of clarified patient plasma for 120 min at RT on a shaking platform
- Wash as above with an additional 5 min final wash
- Incubate membranes in 1:250 dilution of biotin anti-human IgG or IgM for 90 min at RT on a shaking platform
- Wash as above with an additional 5 min final wash
- Incubate membranes in 1:1000 dilution of streptavidin-horseradish peroxidase conjugate for 60 min at RT on a shaking platform
- Wash as above
- Incubate membranes in Diaminobenzidine peroxidase substrate for 5 min to allow colour development, stop reaction by rinsing membrane with water

The results in Figure 3 for patients 112, 305 and 402 clearly show that vaccination over the period of 16 weeks (four to six doses) can result in an increase in antibody titre against cell line lysates and also cross reactivity against lysates not received in this vaccination regime (other than DTH testing).

*(c) Antibody Titre Determination*

Antibody titres were determined by coating ELISA plates with standardised cell line lysates and performing dilution studies on serum from vaccinated patients.

*Method for ELISA with anti-lysate IgG.*

1. Coat plates with 50 μl/well lysates (@10μg/ml) using the following dilutions:-

| Lysate | Protein conc | Coating conc | amount/ml | amount in 5mls μl |
|---|---|---|---|---|
| PNT2 | 2.5 mg/ml | 10 μg/ml | 3.89 μl | 19.4 μl |
| 1542 | 4.8 mg/ml | 10 μg/ml | 2.07 μl | 10.3 μl |
| Du145 | 2.4 mg/ml | 10 μg/ml | 4.17 μl | 20.8 μl |
| LnCap | 2.4 mg/ml | 10 μg/ml | 4.12 μl | 20.6 μl |

2. Cover and incubate overnight @ 4°C
3. Wash x2 PBS-Tween. Pound plate on paper towels to dry.
4. Block with PBS/10%FCS (100μl/well)
5. Cover and incubate @ room temperature (RT) for 1hour (minimum).
6. Wash x2 PBS-Tween
7. Add 100μl PBS-10% FCS to rows 2-8
8. Add 200μl plasma sample (diluted 1 in 100 in PBS-10%FCS ie. 10μl plasma added to 990μls PBS- 10% FCS) to row 1 and do serial 100μl dilutions down the plate as below. Discard extra 100μl from bottom well. Cover and incubate in fridge overnight.
9. Dilute biotinylated antibody (Pharmingen; IgG 34162D) ie. final conc 1mg/ml (ie 20ml in 10mls).
10. Cover and incubate @RT for 45min.
11. Wash x 6 as above.
12. Dilute streptavidin –HRP (Pharmingen, 13047E 0; dilute 1:1000 (ie10ml ->10 mls).
13. Add 100ml/well.
14. Incubate 30 min @RT.
15. Wash x 8.
16. Add 100ml substrate / well. Allow to develop 10-80 min at RT.
17. Colour reaction stopped by adding 100ml 1M $H_2SO_4$.
18. Read OD @ A405nm.

The results in Figure 3 for patients 112, 305 and 402 show antibody titres at baseline (0), 4 weeks, 8 weeks and 16 weeks. The data show that after vaccination with at least four doses, patients can show an increase in antibody titre against cell line lysates and also cross-reactivity against cell lines not received in this vaccination regime (except as DTH doses).

*(d) Evaluation of PSA Levels*

PSA levels for patients receiving the vaccine were recorded at entry into the trial and throughout the course of vaccination, using routinely used clinical kits. The PSA values for patients 110, 303 and 404 are shown in Figure 4 (vertical axis is serum PSA in ng/ml; horizontal axis is time, with the first time point representing the initiation of th vaccination programme) and portray a drop or partial stabilisation of the PSA valu s, which in this group of patients normally continues to rise, often exponentially. The result for patient 110 is somewhat confounded by the radiotherapy treatment to alleviate bone pain, although the PSA level had dropped prior to radiotherapy.

**Example 2: Use of a Normal Melanocyte in a Murine Melanoma Protection Model Model I**

A normal melanocyte cell line was used in a vaccination protection model of murine melanoma utilising the B16.F10 as the challenge dose. The C57 mice received two vaccinations of either PBS, $5x10^6$ irradiated K1735 allogeneic melanoma cells or $5x10^6$ irradiated Melan P1 autologous normal melanocyte cells on days -14 and -7. Challenge on day 0 was with $1 \times 10^4$ B16.F10 cells and tumour volume measured every three days from day 10 onwards. Animals were sacrificed when the tumour had grown to 1.5x1.5 cm measured across the maximum dimensions of the tumour. Figure 5 shows that vaccination with Melan1P cells offer some level of protection against this particularly aggressive murine tumour.

**Claims**

1. An immunotherapeutic agent for the treatment of prostate cancer comprising three human prostate cell lines of which one cell line is derived from normal tissue and the other two cell lines are derived from tumour tissues.

2. An immunotherapeutic agent for the treatment of prostate cancer comprising three human prostate tumour cell lines of which one cell line is derived from a primary tumour and the other two cell lines are derived from two different tumour tissues.

3. An immunotherapeutic agent for the treatment of prostate cancer comprising three human prostate cell lines of which three cell lines are derived from one, two or three normal tissue(s).

4. An immunotherapeutic agent for the treatment of prostate cancer comprising three human prostate cell lines of which two cell lines are derived from normal tissue and the other cell line is derived from a tumour site.

5. An immunotherapeutic agent of claims 1, 3 and 4 where the lines derived from normal tissue are chosen from from PNT1A (ECACC Ref No: 95012614) or PNT2 (ECACC Ref No: 95012613)

6. An immunotherapeutic agent of claims 1, 2 and 4 where the line(s) derived from tumour tissue is/are chosen from NIH1519-CPTX, NIH1532-CP2TX, NIH1535-CP1TX, NIH1542-CP3TX, CA-HPV-10, LnCap, DU145 or PC3.

7. An immunotherapeutic agent for the treatment of prostate cancer comprising three cell lines, namely PNT2, NIH1542-CP3TX andDU145.

8. An immunotherapeutic agent for the treatment of prostate cancer comprising three cell lines, namely PNT2, NIH1542-CP3TX and LnCap.

9. An immunotherapeutic agent for the treatment of prostate cancer comprising three cell lines, namely PNT2, DU145 and LnCap.

10. An immunotherapeutic agent of claims 1-9 wherein the tumour cell lines have been irradiated at 50 to 300 Gy.

11. An immunotherapeutic agent of claims 1-9 wherein the tumour cell lines have been irradiated at 100 to 150 Gy.

12. An immunogenic composition comprising an immunotherapeutic agent of claims 1-11 combined with a vaccine adjuvant selected from mycobacterial preparations such as BCG or M. Vaccae, Tetanus toxoid, Diphtheria toxoid, Bordetella Pertussis, interleukin 2, interleukin 12, interleukin 4, interleukin 7, Complete Freund's Adjuvant, Incomplete Freund's Adjuvant or other non-specific agents adjuvant.

13. An immunogenic composition comprising an immunotherapeutic agent of claims 1-11 combined with a vaccin adjuvant selected from mycobacterial preparations such as BCG or M. Vacca .

14. An immunotherapeutic agent or composition of claims 1-13 wherein the cells are formulated with a cryoprotectant solution including but not limited to 10-30% v/v aqueous glycerol solution, 5-20% v/v dimethyl sulphoxide or 5-20% w/v human serum albumin either as single cryoprotectants or in combination.

15. An immunotherapeutic agent or composition of claims 1-13 wherein the cells are formulated with a cryoprotectant solution including 5-20% v/v dimethyl sulphoxide and 5-20% w/v human serum albumin in combination.

16. An immunotherapeutic agent or composition of claims 1-15 that induces an immune response in patients characterised by activation of immune T-cells.

17. An immunotherapeutic agent or composition of claims 1-15 that induces an immune response in patients characterised by induction of antibody production.

18. An immunotherapeutic agent or composition of claims 1-15 that induces a decrease in the rate of rise or a decline in the level of serum PSA in prostate cancer patients.

19. An immunotherapeutic agent or composition according to claims 1 to 18 that is administered intradermally.

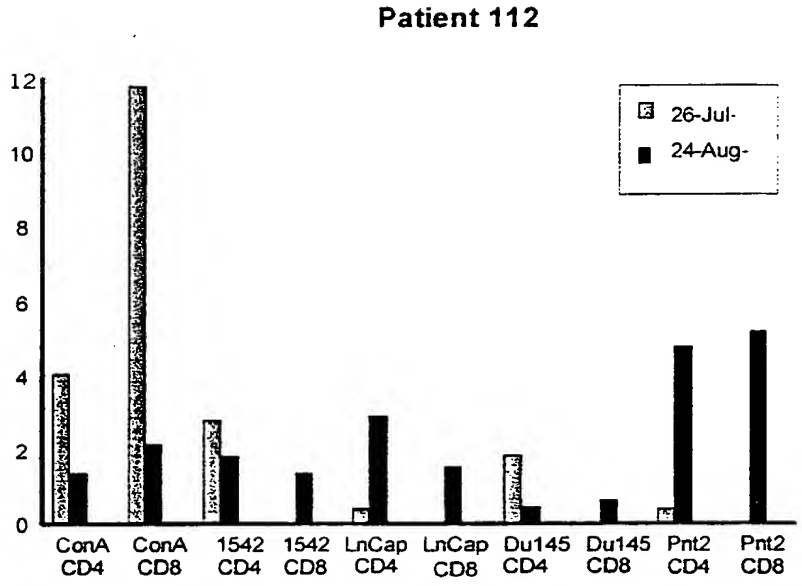
20. An immunotherapeutic agent or composition according to claims 1 to 18 that is administered intra-prostatically.

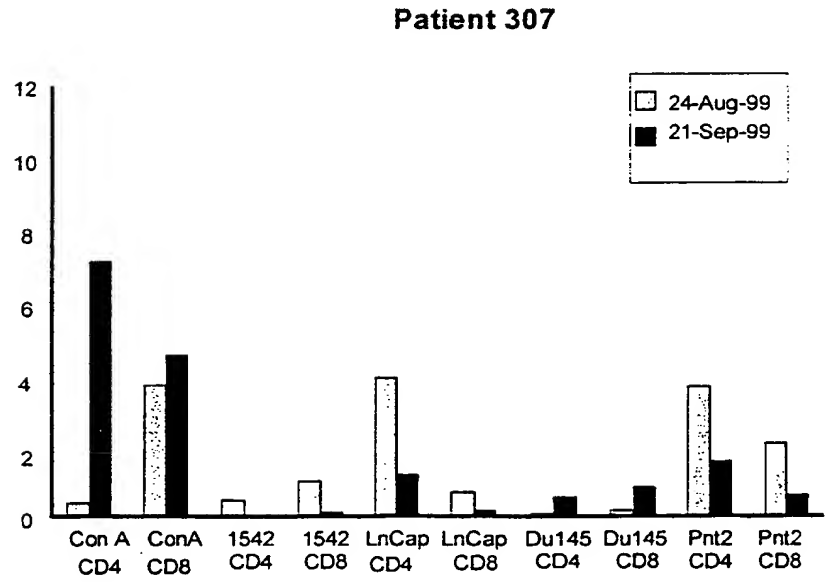
21. A immunotherapeutic vaccine composition for the treatment of prostate cancer, which comprises or consists of an agent according to any preceding claim together with a physiologically acceptable excipient, adjuvant or carrier.

22. A method of prophylaxis or treatment of prostate cancer, which includes administering to a patient an agent or composition according to any preceding claim in one or more doses in suitable dosage form.

23. Use of an agent according to any of claims 1 to 11 in the manufacture of a medicament for the treatment of human prostate cancer.

Figure 1 T Cell proliferation Data for Patients 112, 307 and 406



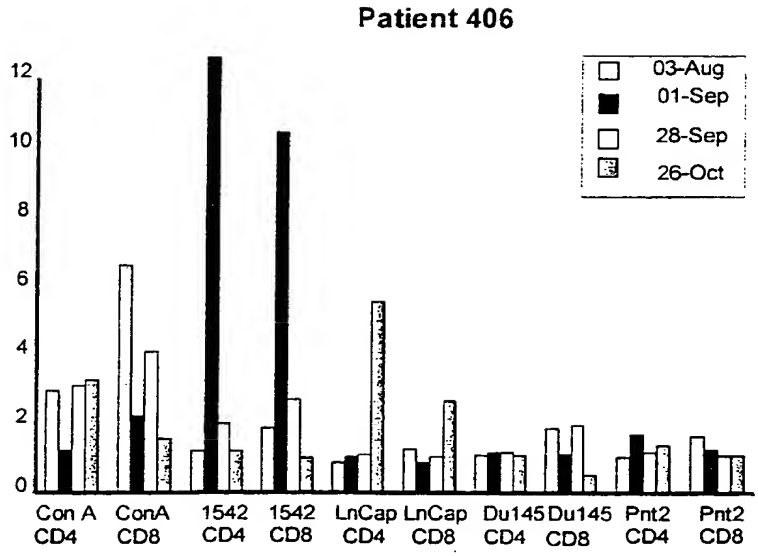
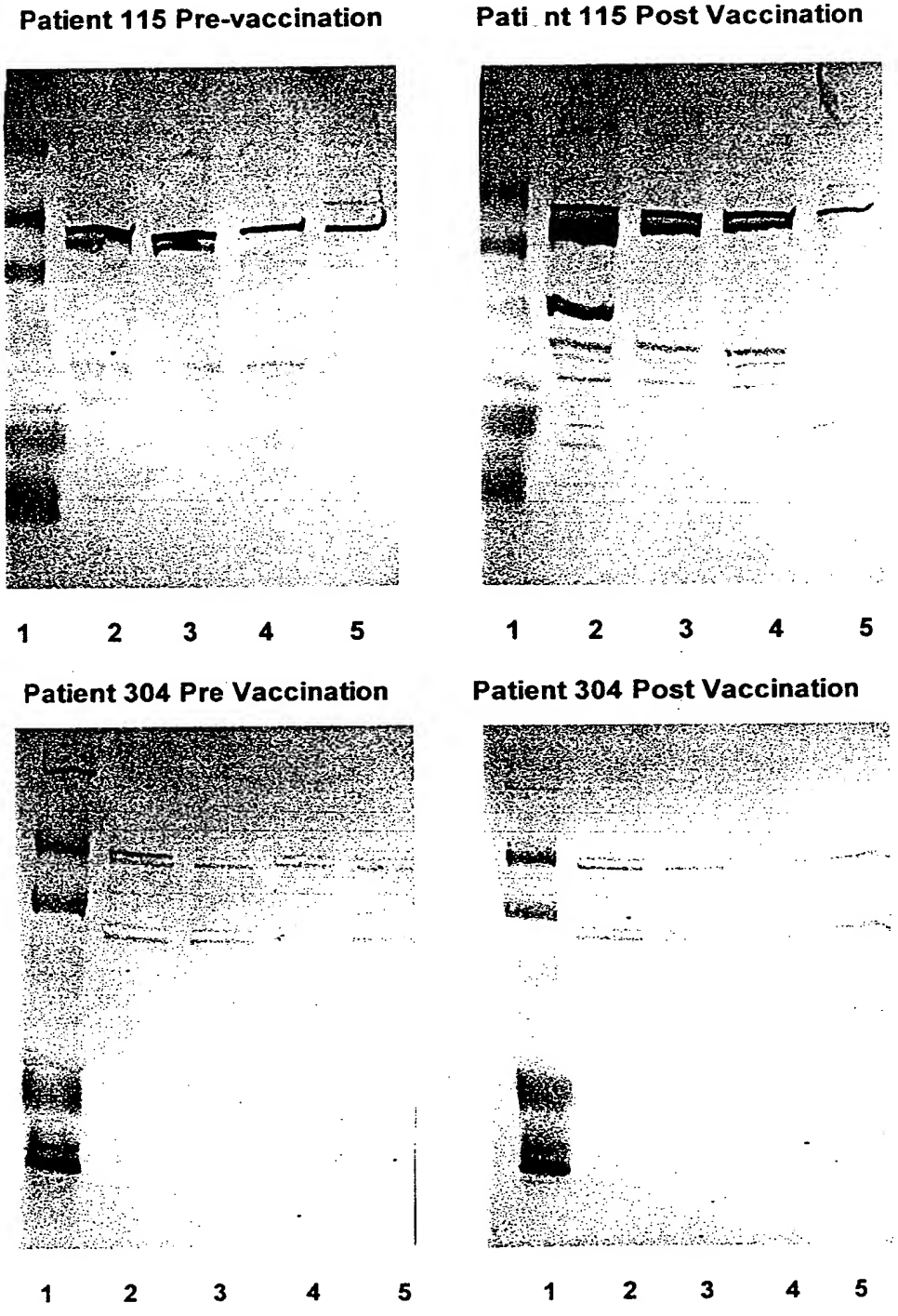
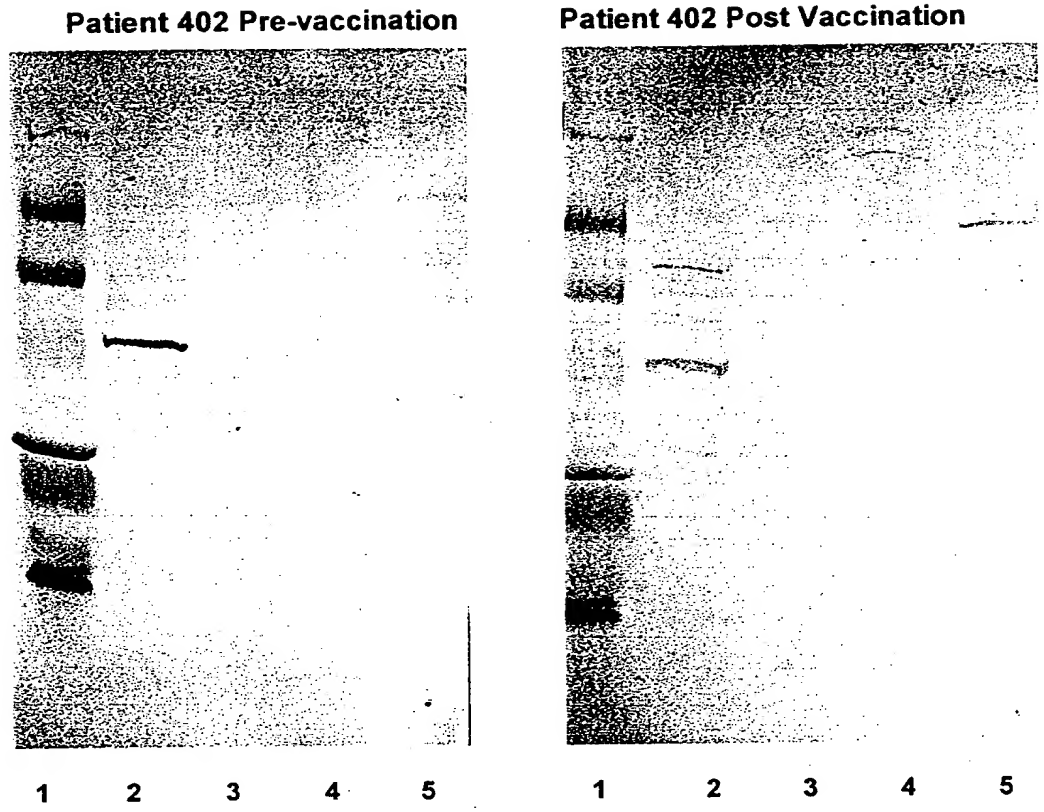

Patient 406
03-Aug
01-Sep
28-Sep
26-Oct
12
10
8
6
4
2
0
Con A CD4
ConA CD8
1542 CD4
1542 CD8
LnCap CD4
LnCap CD8
Du145 CD4
Du145 CD8
Pnt2 CD4
Pnt2 CD8

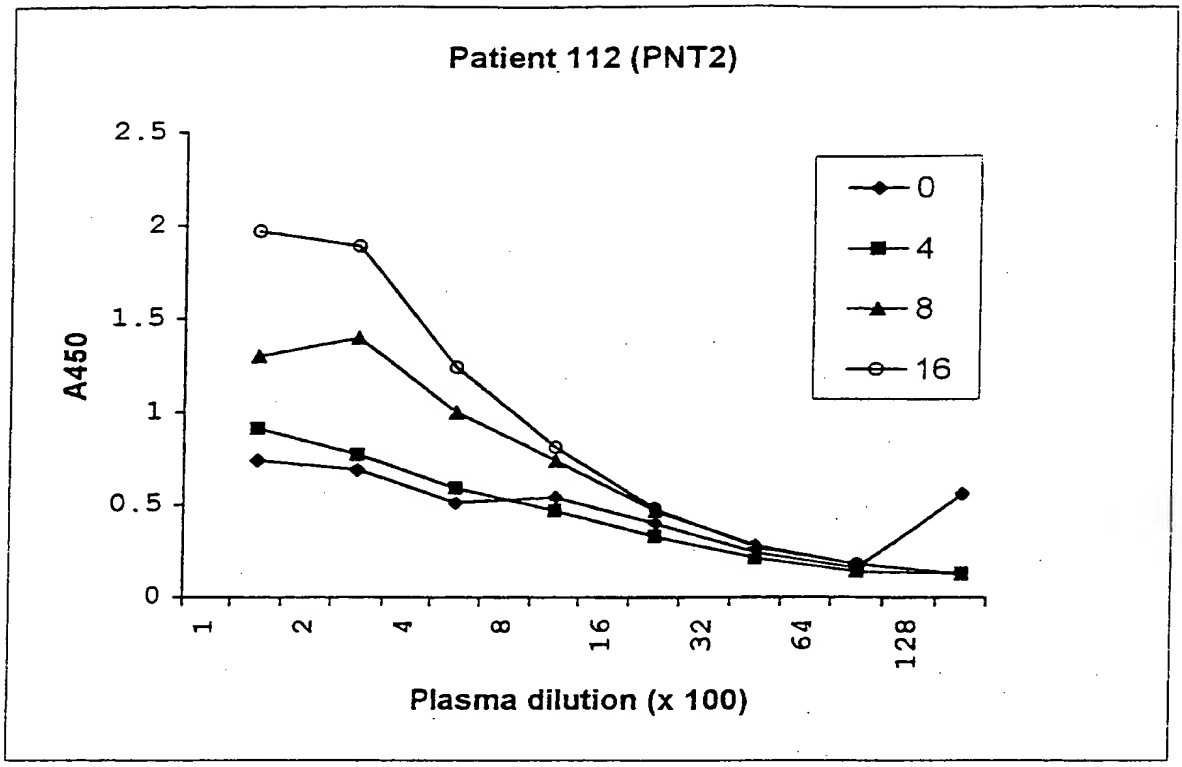
Figure 2 Western Blot Analysis of Serum From Pati nts 115, 304 and 402




1= Molecular weight markers, 2= PNT2 lysate, 3= 1542 lysate, 4=DU145 lysate, 5=LnCap lysate




1= Molecular weight markers, 2= PNT2 lysate, 3= 1542 lysate, 4=DU145 lysate, 5=LnCap lysate


Patient 112 (PNT2)
A450
2.5
2
1.5
1
0.5
0
1
2
4
8
16
32
64
128
Plasma dilution (x 100)
0
4
8
16

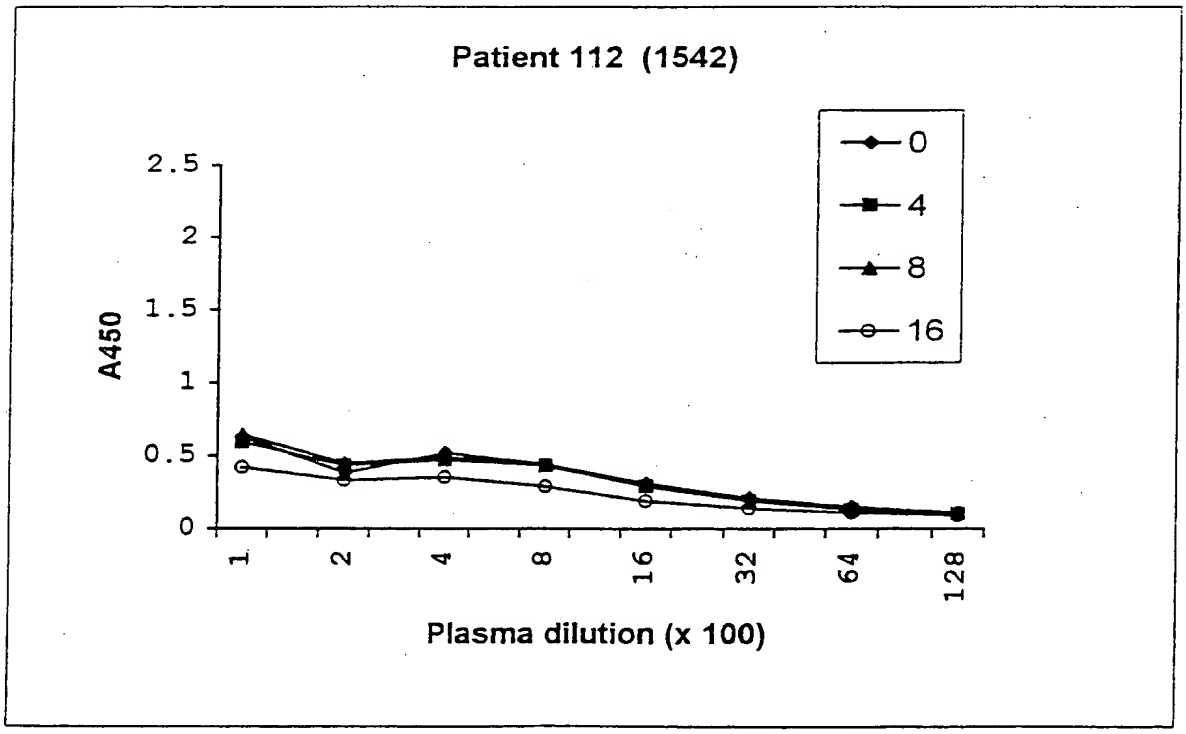
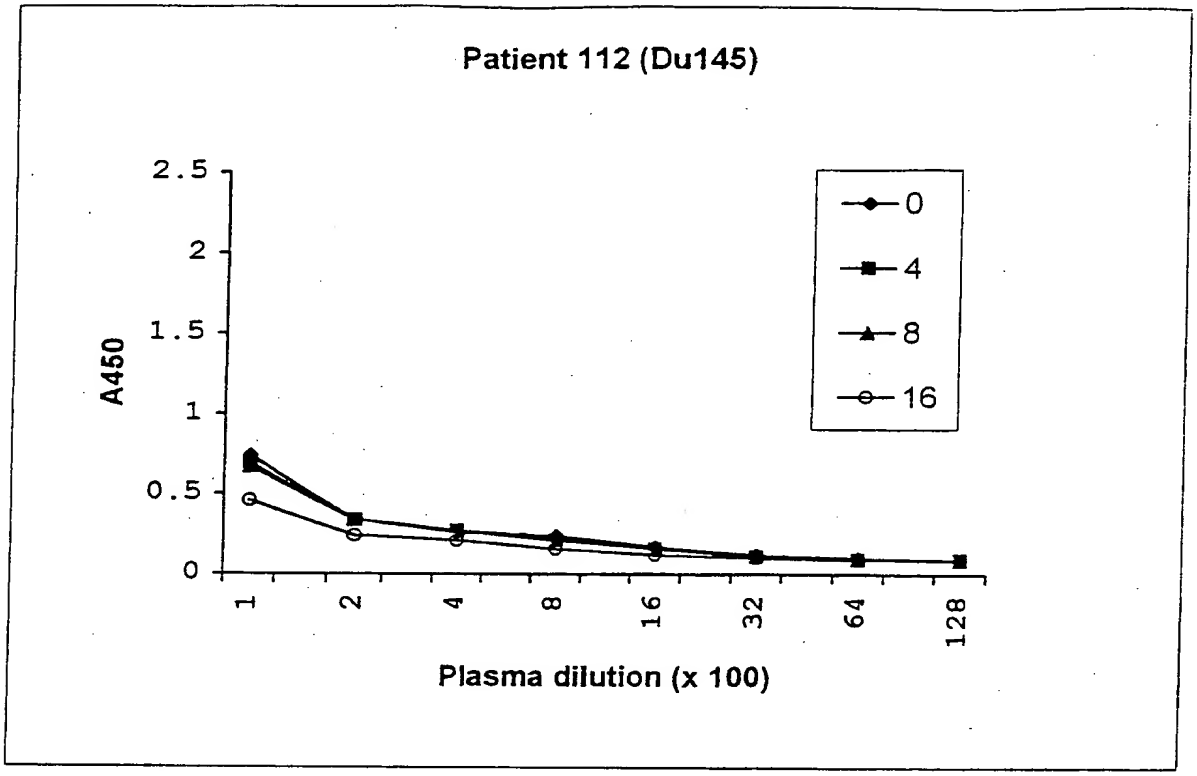

Patient 112 (1542)
A450
2.5
2
1.5
1
0.5
0
1
2
4
8
16
32
64
128
Plasma dilution (x 100)
0
4
8
16

Figure 3



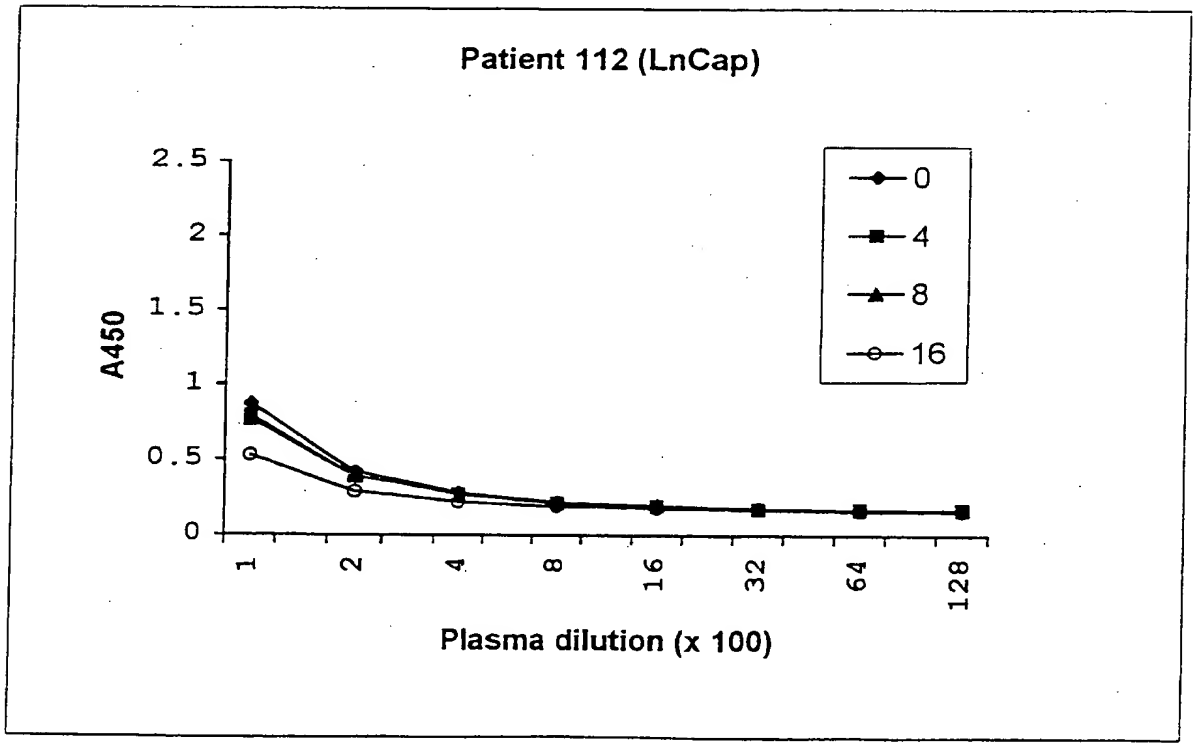
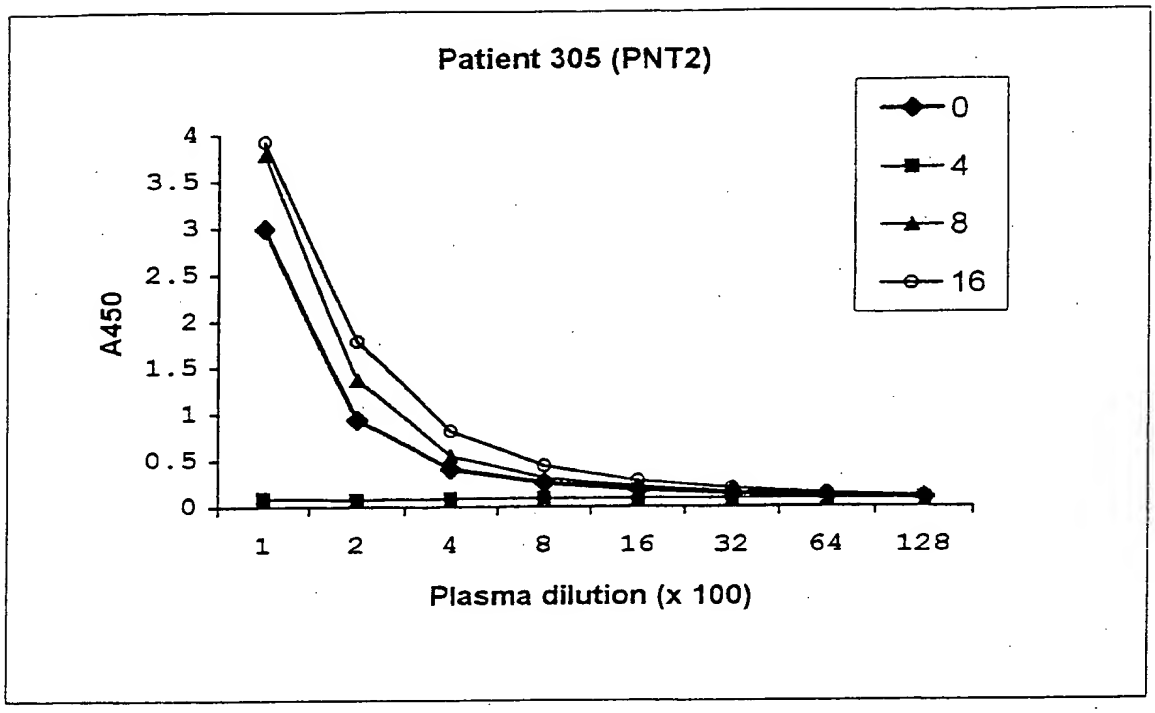




Figure 3 (continued)



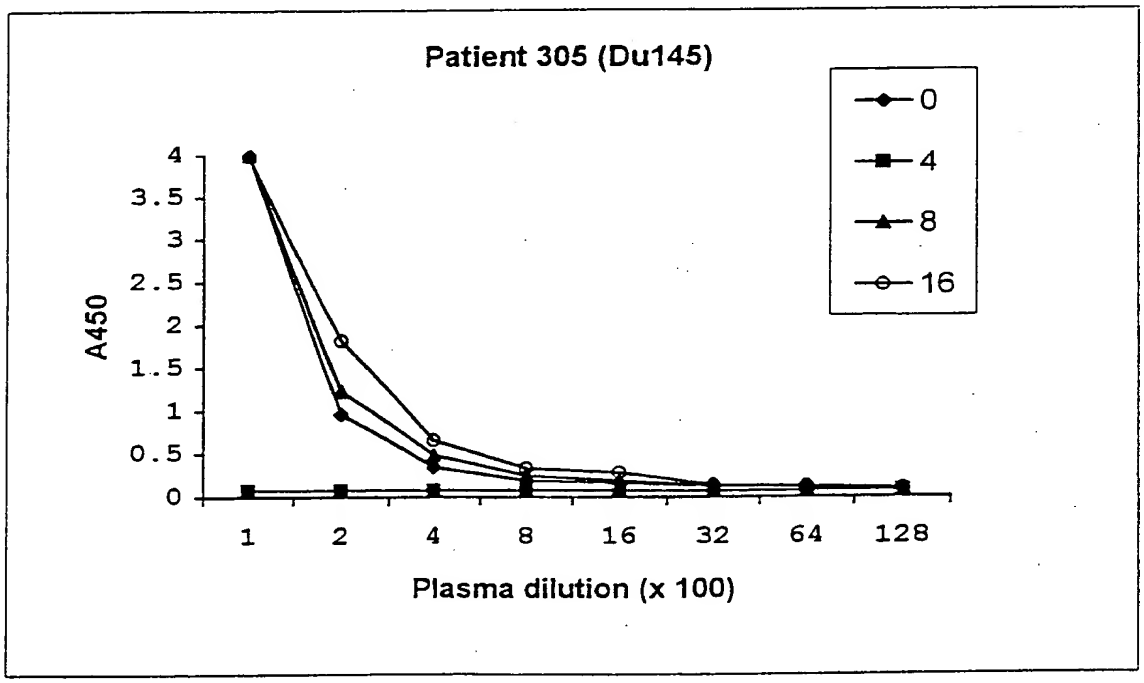
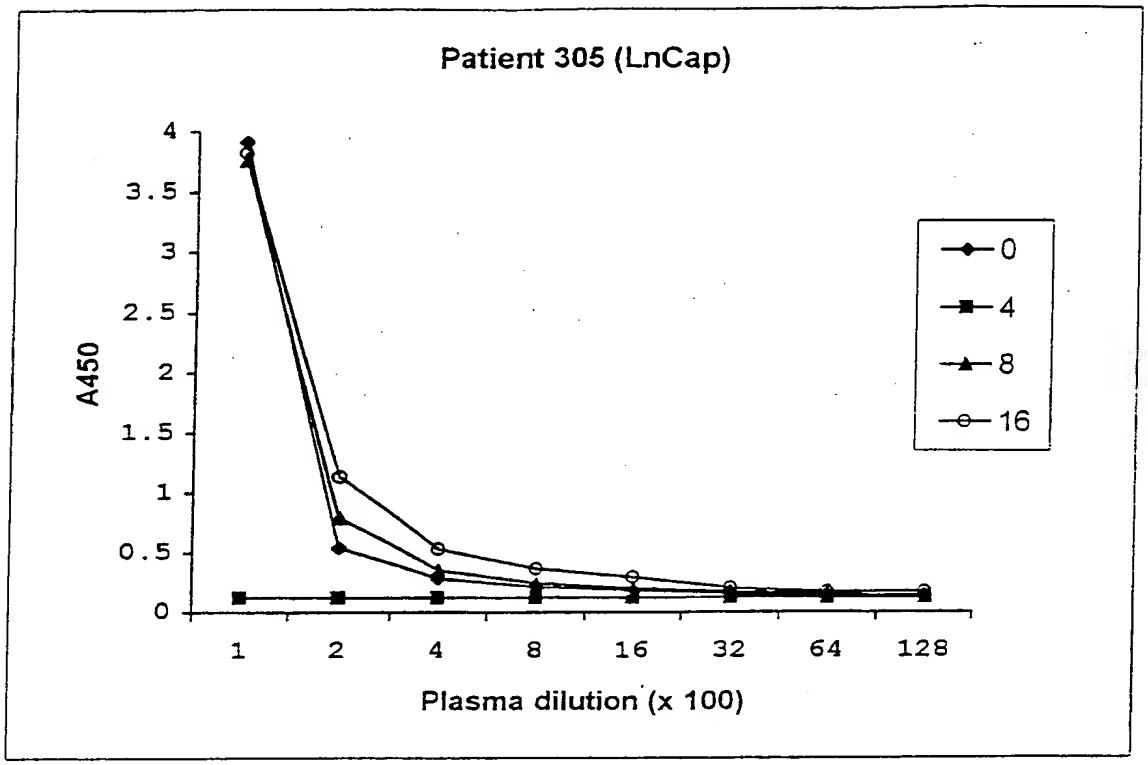




**Figure 3 (continued)**



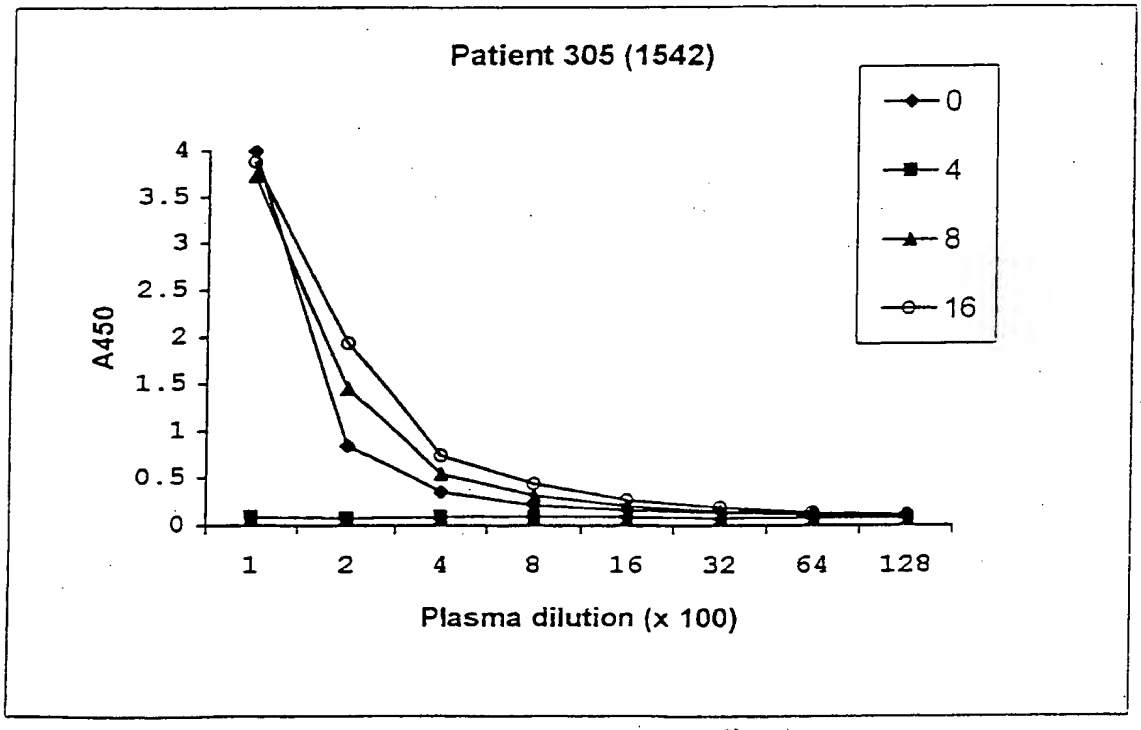
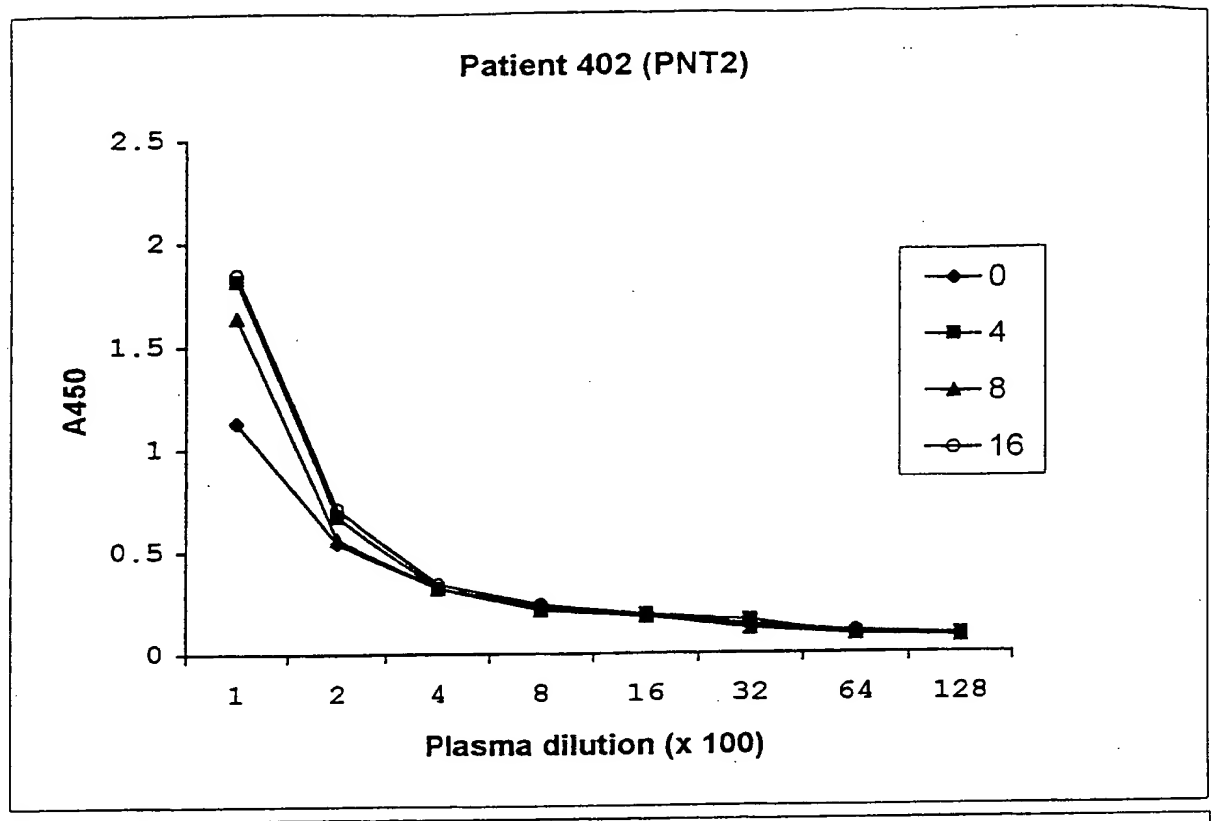




**Figure 3 (continued)**



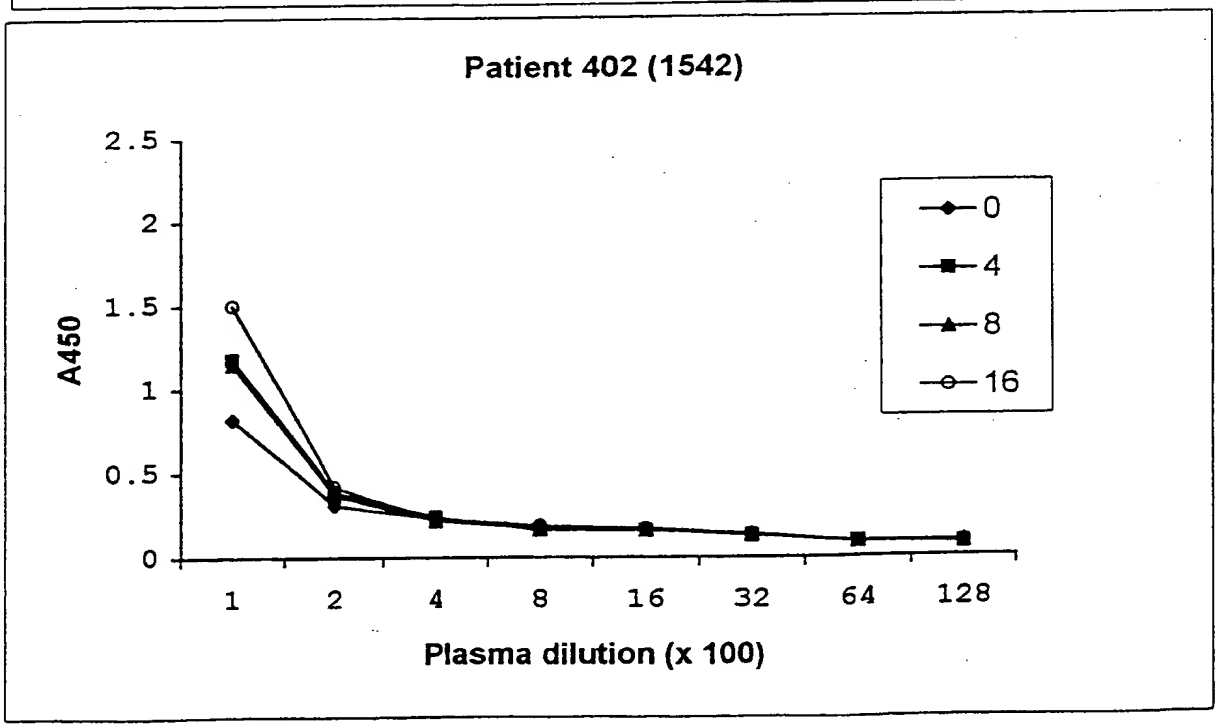
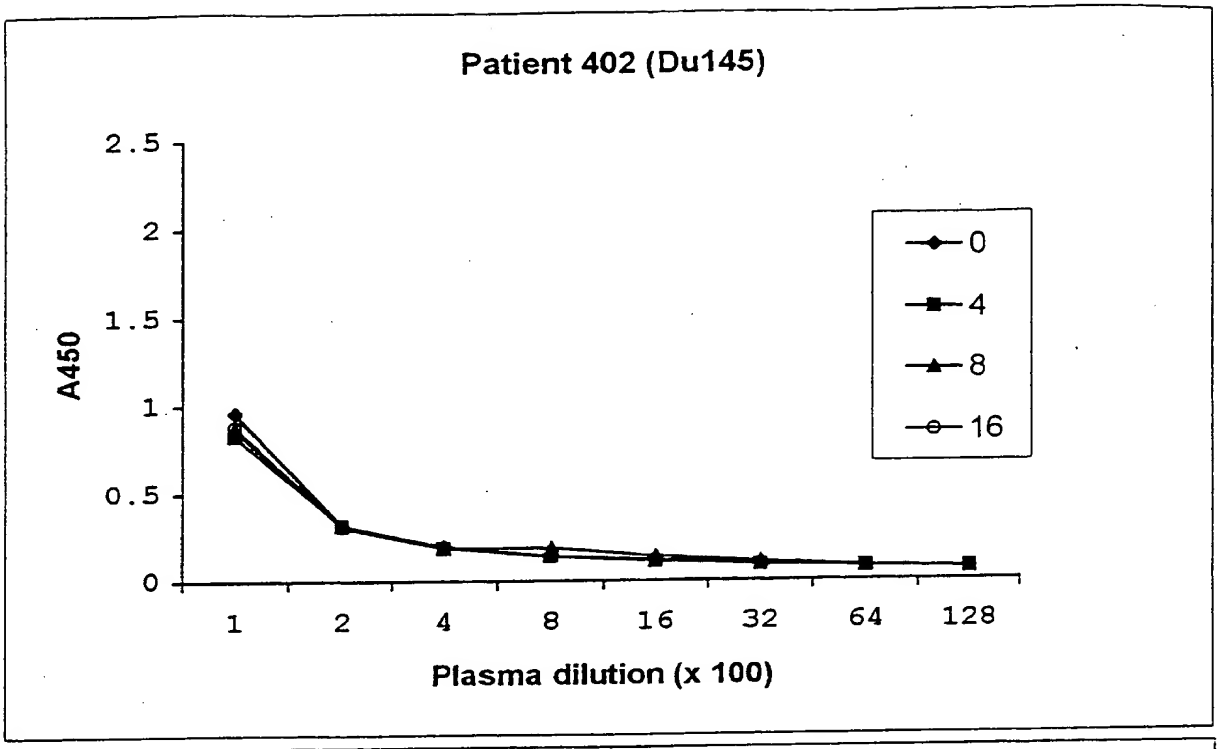




Figure 3 (continued)



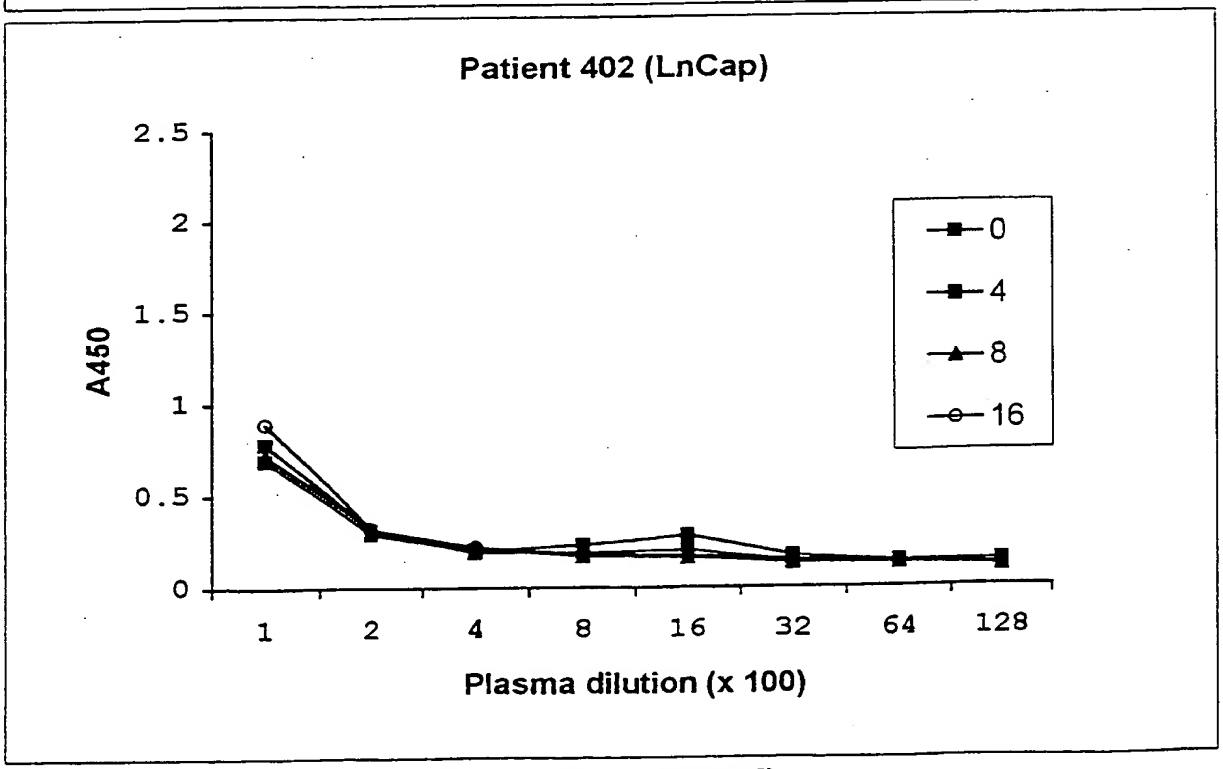
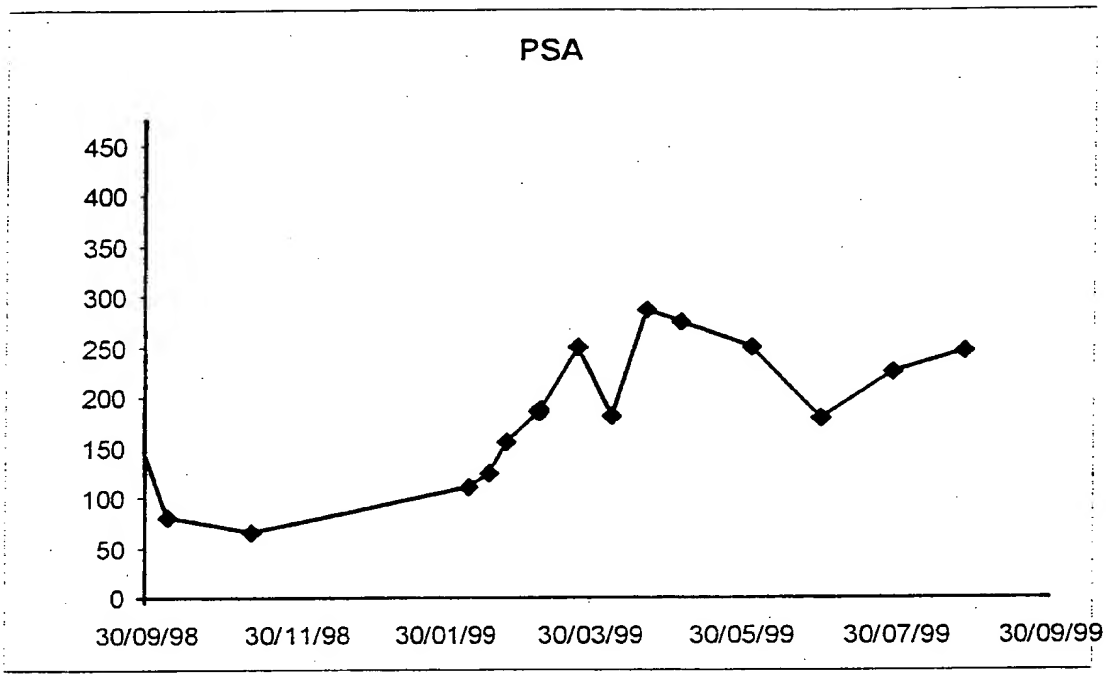



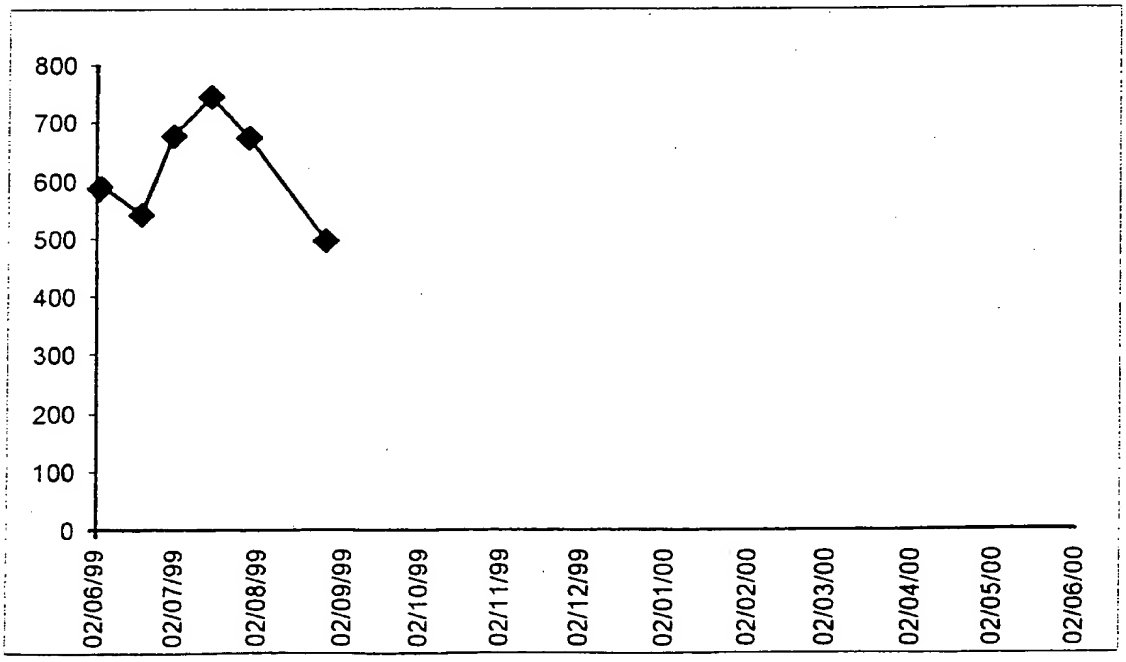

Figure 3 (continued)

Figure 4 PSA Data for Patients 110, 303 and 404

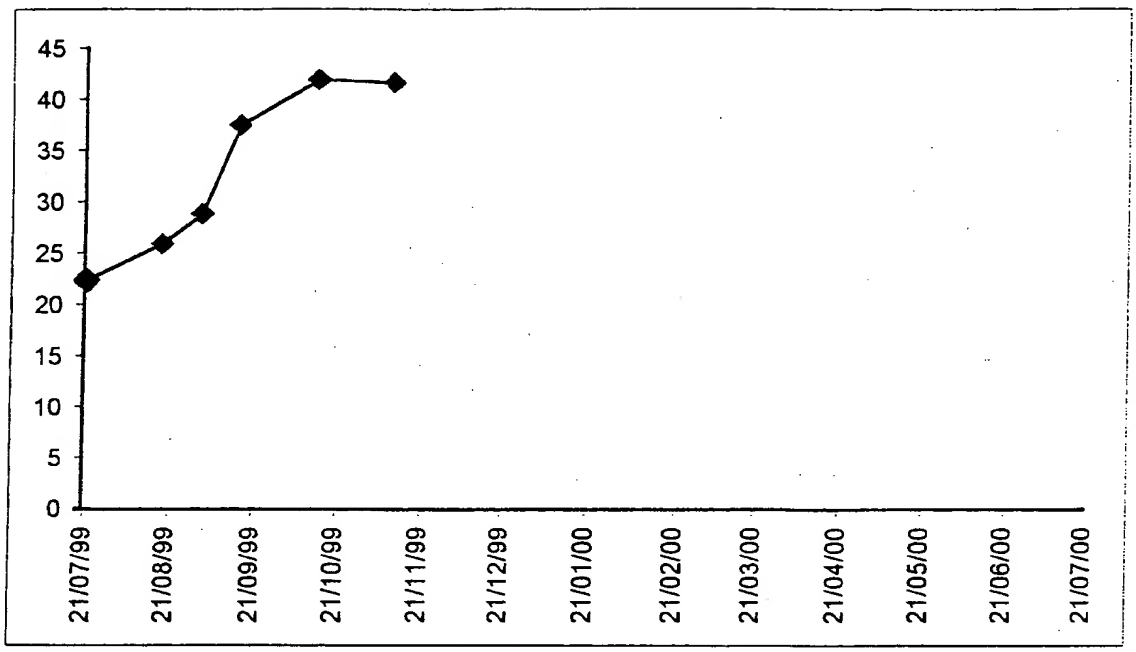
Patient 110




Patient 303



Patient 404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40
35
30
25
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15
10
5
0
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21/08/99
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21/12/99
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21/02/00
21/03/00
21/04/00
21/05/00
21/06/00
21/07/00

**Figure 5 Survival Curves for C57 Mice Immunised With Normal Melanocytes**